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S p e c i f i c a t i o n

Be It Known That We, MICHAEL A. MURPHY a citizen of Ireland, resident of La Jolla, County of San Diego, State of California and MITCHELL R. MALACHOWSKI a citizen of the United States, resident of San Diego, County of San Diego, State of California, have invented a new and useful

COMPOSITION, SYNTHESIS AND THERAPEUTIC
APPLICATIONS OF POLYAMINES

of which the following is a specification:

10017235-121801

Composition, Synthesis and Therapeutic Applications of Polyamines

PRIOR APPLICATION

5 This is a continuation-in-part of copending application serial number 09/486,310 filed February 23, 2000, issued from PCT application serial number US98/17301 filed August 21, 1998 a continuation of application serial number 08/915,660 filed August 21, 1997, now patent No. 5,906,996.

FIELD OF INVENTION

10 This invention relates to a process of synthesis and composition of open ring, closed ring, linear branched and or substituted polyamines for the treatment of neurological, cardiovascular, endocrine and other disorders in mammalian subjects, and more specifically to the therapy of 15 Parkinson's disease, Alzheimer's disease, Lou Gehrig's disease, Binswanger's disease, Olivopontine Cerebellar Degeneration, Lewy Body disease, Diabetes, Stroke, Atherosclerosis, Myocardial Ischemia, Cardiomyopathy, Nephropathy, Ischemia, Glaucoma, Presbycusis and Cancer.

CHEMICAL AND THERAPEUTIC BACKGROUND

Chemistry

There are two groups of polyamines described in herein, those derived from 1,3-bis-[(2'-aminoethyl)-amino]propane (2,3,2-tetramine) and those from the macrocycle 1,4,8,11-tetraazacyclotetradecane (cyclam). Of the collection of compounds described, most are not presently known but a few have been prepared previously.

Inherited and Acquired Mitochondrial DNA Damage

Individuals carrying mild mitochondrial DNA base substitutions manifest late onset diseases like Parkinson's and Alzheimer's diseases and familial deafness, whereas persons with moderately deleterious base substitutions develop Type II diabetes, Leber's Hereditary Optic Neuropathy, Myclonic Epilepsy and Ragged Red Fiber Disease (MERRF). Individuals with severely deleterious base substitutions develop pediatric onset myopathies, dystonias and Leigh's syndrome. Wallace D.C. (1992 a,b) suggests that aging and common degenerative diseases result from energetic decline caused by inherited oxidative phosphorylation (OXPHOS) gene defects and acquired somatic mutations. Mild mitochondrial deoxyribonucleic acid (DNA) rearrangements and duplications cause maternally inherited adult-onset diabetes and deafness. More severe rearrangements and deletions have been associated with adult-onset Chronic Progressive External Ophthalmoplegia (CPEO) and Kearns-Sayre Syndrome (KSS) and Pearson's Marrow / Pancreas Syndrome. Primary oxidative phosphorylation (OXPHOS) diseases frequently have a delayed onset, organ

selectivity and an episodic, progressive course. For example the A3243G mutation associated with mitochondrial encephalopathy, lactic acidemia, stroke-like episodes (MELAS) can pure a pure cardiomyopathy, pure diabetes and deafness, or pure external ophthalmoplegia (Naviaux R.K. 2000).

The level of oxidative damage to mitochondrial and nuclear DNA, as measured by 8-hydroxy-2'-deoxy guanosine increases with age (Mecocci P. et al 1993) and oxidative damage to mitochondrial DNA occurs Alzheimer's disease (Mecocci P. et al 1994 and 1998).

Some organs may be more prone to oxidative damage due to lack of protective substances, for example uric acid an antioxidant and transition metal chelator (Ames B.N. et al 1981) is not present in brain that may limit recovery from ischemic reperfusion damage and metal accumulation post stroke.

Examples of Diseases Where Mitochondrial DNA Malfunctions

In Parkinson's disease reduced glutathione is depleted due to loss of endogenous polyamines, thus reducing the activity of glutathione peroxidase and permitting oxidative damage. Oxidative damage disintegrates mitochondrial DNA into hundreds of types of mitochondrial DNA fragments which causes release of apoptotic factors and cell death (Ozawa T. et al 1997).

Mitochondrial DNA deletions in brain tissue also increase with age and the increase varies from one brain region to another (Corral-Debrinski M. et al 1992), deletions being highest in the substantia nigra and striatum (Soong N.W. et al 1992) and is also regionally distributed in Alzheimer's disease (Corral-Debrinski M. et al 1994). Environmental agents and nuclear gene defects may cause mitochondrial diseases by predisposing to multiple

mitochondrial DNA deletions or quantitative depletions of mitochondrial DNA content. A reversible depletion of mitochondrial DNA occurs during zidovudine (AZT) therapy (Arnaudo E. et al 1991). Adriamycin inhibits mitochondrial cytochrome c oxidase (COX II) gene transcription leading to cardiomyopathy (Papadopoulou L.C. et al 1999). Mendelian traits causing qualitative and quantitative changes in mitochondrial DNA have been observed (Zeviani M. et al 1995). Nuclear recessive factors can also affect mitochondrial translation and cause age-related respiration deficiency (Isobe K. et al 1998). Wolfram syndrome can be caused by either a mitochondrial or nuclear gene defect (Bu X. et al 1993).

Mitochondrial disorders with neurologic manifestations include; Ptosis, ophthalmoplegia, exercise intolerance, fatigability, myopathy, ataxia, seizures, myoclonus, stroke, optic neuropathy, sensorineural hearing loss, dementias, peripheral neuropathy, headache, dystonia, myelopathy. Mitochondrial disorders with systemic manifestations include; cardiomyopathy, cardiac conduction defects, short stature, cataract, pigmentary retinopathy, metabolic acidosis, nausea and vomiting, hepatopathy, nephropathy, intestinal pseudo-obstruction, pancytopenia, sideroblastic anemia, diabetes mellitus, exocrine pancreatic dysfunction and hypoparathyroidism.

DNA Damage in Neurodegenerative Disorders

Mitochondrial DNA is not protected by histones and lacks a pyrimidine dimer repair system (Clayton DA et al 1974). Mitochondrial DNA has a relatively short half life of six to ten days compared with an up to one month half life of nuclear DNA. The error insertion frequency of polymerase γ is approximately 1 in 7,000 bases, leading to 2-3 mismatched nucleotides per cycle of replication. Hypoxia induces damage to nuclear DNA and to a greater

extent to mitochondrial DNA (Englander E. et al 1999). Nuclear and mitochondrial DNA
 repair declines during aging in neurons and in cortical glial cells (Schmitz C. et al 1999). 8-
 hydroxyguanosine (8-OHG) immunoreactivity is increased in the substantia nigra, nucleus
 raphe dorsalis and oculomotor nucleus of Parkinson's disease patients, and 8-OHG
 immunoreactivity is also increased in the substantia nigra of Olivopontine cerebellar
 degeneration (OCD or MSA) and Lewy body disease patients. Lewy bodies were proposed to
 be degenerating mitochondria (Gai W.P. et al 1977). Mitochondria partially though not
 completely repair DNA damage caused by bleomycin (Shen C. 1995). Polyamines promote
 repair of Xray induced DNA strand breaks (Snyder R.D. 1989). Polyamine depletion caused
 by α -difluoromethylornithine (DFMO) increases the number of strand breaks caused by 1,3-
 bis(2chloro-ethyl)-1-nitrourea (BCNU) (Cavanaugh P.F. et al 1984). Physiological
 concentrations of spermine and spermidine prevent single strand DNA breaks induced by
 superoxide (1O_2) (Khan A.U et al 1992). L-DOPA and Cu(II) generate reactive oxygen
 species, conversion of guanine to 8-hydroxyguanine and cause strand breakage of DNA
 (Husain S. et al 1995). The metal catalyzed oxidation of dopamine and related amines to
 quinones and semiquinones occurs during pigment deposition and may precipitate cellular
 damage in Parkinson's and Lou Gehrig's diseases (Levy G. et al 1997). Melanin in
 association with Cu(II) is also capable of causing DNA strand breakage (Husain S. et al 1997).
 Copper concentrations in the cerebrospinal fluid of Alzheimer's patients is increased 2.2 fold
 and caeruloplasmin concentrations is also increased (Bush A.I. et al 1994). Copper
 concentrations are elevated to 0.4 mM and iron and zinc to 1 mM in the neuropil of
 Alzheimer's brain (Lovell M. et al 1998, Smith M.A. et al 1997).
 Mitochondrial DNA content is depleted in Parkinsonian brain and following MPTP
 administration in experimental animals due to deficient DNA replication in both instances

(Miyako K. et al 1997 and 1999). MPP+ destabilizes D-loop structure thereby inhibiting the transition from transcription to replication of mitochondrial DNA (Umeda S. et al 2000).

Alzheimer's disease patients brains have decreased levels of mitochondrial DNA, increased levels of 8-OHdeoxyguanosine and increased DNA fragmentation (de la Monte S.M. et al 2000). Increased levels of point mutations, for example at nucleotide pair 4366 in the tRNA^{GLN} gene was observed (Shoffner J.M. et al 1993). The risk of Alzheimer's disease increases when a maternal relative is afflicted with the disease (Duara R. et al 1993, Edland S.D. et al 1996).

DNA damage was proposed as a cause of Lou Gehrig's disease by Bradley W.G et al and deficiency of cytochrome c oxidase activity and a cytochrome c microdeletion were observed by Borthwick G.M. et al (1999) and Comi G.P. et al (1998).

A decreased activity of mitochondrial complex IV and citrate synthase was observed in Olivopontine Cerebellar Degeneration (OCD or MSA) (Schapira A.H.V. 1994, 1998).

Biological Actions of Polyamines that Maintain Brain Function and Prevent Neurodegeneration

However the pathology of several disease states which are described below involves more than the initial DNA damage and correspondingly the influence of therapeutic agents in these diseases involves control of DNA damage and other cellular injuries simultaneously.

I previously reported the ability of 2,3,2 tetramine in Murphy U.S. Patent No. 5,906,996 to prevent MPTP induced dopamine loss and the applicability of such compounds in the treatment of neurodegeneration, this being notated herein in its entirety by this reference.

A model of neurodegeneration involving Parkinson's, Alzheimer's, Olivopontine Cerebellar Degeneration, Lewy Body, Binswanger's and Lou Gehrig's diseases involving a similar constellation and cascade of events, whereby the final disease is determined by the duration of damage and the anatomical distribution of damage was described. The principal highlights of this pattern of neurodegeneration and its treatment by polyamines are summarized as follows:

The Neurodegenerative Pathway In Parkinson's, Olivopontine Cerebellar Atrophy (MSA), Alzheimer's, Lewy Body, Binswanger's and Lou Gehrig's Diseases

There are five principal aspects of neuronal damage in this pattern of neurodegeneration all of which are prevented by optimized polyamine molecules; Mitochondrial DNA Damage, Growth Factor Functions, Receptor Activities, Energetics and Redox Homeostasis and Deposition of Amyloid.

Cascade of Events in the Pathogenesis of Neurodegeneration:

Mitochondrial DNA is damaged by dopamine and xenobiotics in the presence of reduced levels of naturally occurring polyamines.

Polyamines competitively block the uptake of xenobiotics which depigment pigment. Depigmentation releases organic molecules and free metals which damage mitochondrial DNA bases. Polyamines protect DNA from damage by organic molecules by steric interactions (Baeza I. et al 1992). They sequester the metals directly and induce transcription of metallothionein (Goering P.L. et al 1985), the metals being catalytic in reactions damaging

DNA bases. They also induce transcription of growth factors such as nerve growth factor, brain derived neuronotrophic factor (Chu P. et al 1995, Gilad G. et al 1989. Polyamines regulate the activity N-methyl-d-aspartate (NMDA) receptor and affect the level of agonism or antagonism at the MK801 ion channel (Beneviste M. Et al 1993, McGurk J.F. 1990) and the activity of protein kinase C (Mezzetti G et al 1988, Moruzzi M.S. et al 1990, 1995).

Polyamines regulate redox homeostasis by binding glutathione (Dubin D.T. 1959). These primary deficits associated with polyamine deficiency cause the neuronal dedifferentiation processes of these diseases via the changes in growth factor levels or ratios, the rapid entry of calcium via the MK801 ion channel and the metabolic consequences by damaged RNA transcripts causing production of defective cytochromes.

Secondarily defective cytochromes are proteolysed and release enkephalin by products and also release free iron into the mitochondrial matrix. The iron is leached from damaged calcium laden mitochondria into the cytosol of the neurons. NMDA receptor activation causes excess calcium entry into cells.

Thirdly gross elevation of the free level of a metal such as iron causes displacement of other metals such as copper, nickel, cobalt and lead from sites where they are bound. One or more of these metals overactivate preasapate proteases (Abraham 199a, 199b, 1992, Black 1989, Blomgren 1989, Chakrabarti 1989, Dawson 1987, Dawson 1988, Edelstein 1988, Hamakubo 1986, Koistra 1984, Matus 1987, Perlmutter L.S. et al 1988, Press E.M. 1960, Rabbazoni B.L. 1992, Rose C. 1988, Rose C. 1989, Scanu A.M. 1987, Whitaker J.N. 1979) which can produce β -amyloid and tangle associated proteins. In Parkinson's Disease and Alzheimer's Disease there is an increase in free copper levels in the absence of an absolute increase in copper levels or more likely an actual decrease in total tissue copper levels due to its loss in the cerebrospinal fluid. The free copper will activate amine oxidase, tyrosinase,

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copper zinc superoxide dismutase and monoamine oxidase B. The preaspartate proteases may be activated by several divalent metal ions including such as zinc, iron, calcium, cobalt. The literature on these proteases indicates that zinc and calcium and copper are particularly likely. Given a role for divalent metals in activating preaspartate proteases and amyloid production as a tertiary event in this model, it is in concordance with the clinical situation whereby patients present with Parkinson's Disease and subsequently Alzheimer's Disease rather than the converse. In Guamanian Parkinsonian Dementia the plaque formation likewise follows motor neuron and Parkinsonian pathology after many years or decades.

More specifically, therapeutic polyamine compounds like 2,3,2-tetramine have multiple actions on this cascade of events extending from DNA damage to amyloid production;

a) Competitive inhibition of uptake of xenobiotics at the polyamine transport site, such organic molecules being a cause of depigmentation and DNA damage; b) Steric shielding of DNA from organic molecules by compacting DNA; c) Limitation of mitochondrial DNA damage by removal of free copper, iron, nickel, mercury and lead ions by the presence of a polyamine; d) Induction of metallothionein gene transcription; e) Induction of nerve growth factor, brain derived neuronotrophic factor and neuronotrophin-3 gene transcription; f) Regulation of affinity of NMDA receptors and blockade of the MK801 ion channel; g) Inhibition of protein kinase C; h) Mitochondrial reuptake of calcium; i) Binding and conservation of reduced glutathione; j) Induction of ornithine decarboxylase by glutathione; k) Maintenance of the homeostasis of the redox environment in brain; l) Non toxic chelation of divalent metals in brain; m) Regulation of activity of preaspartate proteases; n) Inhibition of acetylcholinesterase and butyrylcholinesterase; o) Blockade of muscarinic M₂ receptors; p) Maintenance of ratio of membrane phosphatidylcholine: phosphatidylserine ratio; q)

Inhibition of superoxide dismutase, amine oxidase, monoamine oxidase B by binding of free copper; r) Regulation of brain polyamine levels in dementias with maintenance of endogenous polyamine levels; s) Blockade of neuronal n and p type calcium channels.

5 Successful therapy must prevent glutathione loss, prevent mitochondrial DNA damage or cytochrome enzyme malfunction, prevent release of metals including calcium from mitochondria, NMDA receptor blockade, prevent hyperpigmentation and ensuing depigmentation, prevent oxidative enzyme and amyloid producing enzyme activation. Polyamines compounds described herein uniquely have the relevant profile of the above actions and prevent MPTP induced dopamine loss in an animal model.

10 Because none of the changes in Parkinson's or Alzheimer's diseases are pathognomic and because of the overlapping sets of mitochondrial and cytosolic events in Parkinson's disease, Guamanian Parkinsonian dementia, Alzheimer's disease, Binswanger's diseases, Lewy body disease, hereditary cerebral hemorrhage - Dutch type, Olivopontine cerebellar atrophy and Batten's Disease it is anticipated that these compounds will be beneficial in controlling dementia development. The major pathological difference between Parkinson's and Alzheimer's pathological features being the presence of amyloid in Alzheimer's disease and the diseases being closely interlinked by the evolution of Parkinson's disease into Alzheimer's disease with amyloid deposition as the former progresses. At post mortem forty percent of Parkinson brains have amyloid deposits.

The Neurodegeneration Process – Prevention & Treatment by Polyamines:

The following summarizes the principal concurrent and sequential components of neurodegeneration in Parkinson's, Alzheimer's and Lou Gehrig's diseases, the sites of cellular damage and the pivotal relationship between neurotoxins and polyamines in precipitating and preventing neurodegeneration.

Excessive exposure to xenobiotic molecules that migrate into the cell across the polyamine transport pump initiate depolymerization of pigment. During depigmentation more organic molecules and stored heavy metals are released intracellularly. The excessive exogenous (xenobiotics) and endogenous quinones and semiquinones (neurotransmitter by products) organics mutate mitochondrial DNA bases randomly when catalyzed by heavy metals.

When mitochondrial DNA is damaged, the cytochrome proteins produced are dysfunctional. Breakdown of these proteins releases iron intramitochondrially and subsequently intracellularly. The inactive cytochromes fail to produce the energy storage compound adenosine triphosphate (ATP) which operates the cell's various metabolic processes.

The metals released from the pigment and the iron from the mitochondria activates various enzymes including amine oxidase that breaks down polyamines and preaspartate proteases that produce amyloid from its precursor protein. Decreasing polyamine levels below a threshold level by excessive amine oxidase activity results in a positive feedback cycle of further polyamine loss because polyamines bind and conserve the peptide glutathione (GSH) that stimulates the rate limiting enzyme of polyamine production, ornithine decarboxylase.

As well as regulating the inflow and outflow of xenobiotics and binding of toxic free metals, polyamines also compact mitochondrial DNA that is not coiled or supercoiled like nuclear DNA; they promote transcription of several neuronal growth factors; they regulate the activities of several cell surface receptor systems including the n-methyl-d-aspartate (NMDA) receptor. All of these components of neurodegeneration can be controlled using an optimized polyamine.

Peripheral Neuropathy

Peripheral neuropathy occurs in association with mitochondrial encephalomyopathies (Chu C. et al 1997). Vacuolar degeneration of dorsal root ganglia cells may consist of degenerating mitochondria. Mitochondrial DNA mutations may be caused by lipid peroxidation. α -lipoic acid affected improvement in streptozotocin-diabetic neuropathy (Low P.A. et al 1997). Glutathione treats experimental diabetic neuropathy (Brabenboer B. et al 1995).

Probucol and Vitamin E improve nerve blood flow and electrophysiology (Cameron N.E. et al 1994, Karasu C. et al 1995). Hydroxytoluene and carvidilol were also effective in preventing damage in diabetic neuropathy (Cameron N.E. et al 1993 and Cotter M.A. et al 1995).

Optic Neuropathy

Optic neuropathy occurs in multiple sclerosis patients and occasionally these multiple sclerosis patients have LHON associated mitochondrial DNA mutations.

Optic neuroapthy also occurs from toxic exposure to tobacco and methanol as in Cuban epidemic optic neuropathy (CEON) (Sadun A. and Johns D.R. et al 1994). Methanol leads to formate production that inhibits cytochrome oxidase and adenosine triphosphate production is diminished. Decrease in ATP results in decreased mitochondrial transportation and shutdown of axonal transportation.

Glaucoma

In glaucoma the M ganglion cells of the retina degenerate and there is defective axoplasmic flow (Quigley H.A. 1995). Glutamate is elevated in the vitreous body of glaucoma patients (Dreyer E.B. et al 1996), glutamate being more toxic to M ganglion cells (Dreyer M. et al 1994).

The excitotoxic cascade caused by NMDA receptor activation in the optic nerve results in excess calcium influx, increased nitric oxide synthesis and production of oxygen free radicals (Sucher N.J. et al 1997).

Diabetes Mellitus

Mitochondrial DNA content in peripheral blood was observed to be 35% lower in Non Insulin Dependent diabetics (NIDDM) than in controls Lee H.K. et al 1998) and the decline precedes the onset of diabetes. Reduced oxidative disposal of glucose results in insulin resistance in skeletal muscle and / or defective insulin secretion in pancreatic islets. Decreased mitochondrial DNA content impairs fat oxidation in the presence of increased fatty acid

availability, fatty acyl CoA accumulates in the cytosol and thus causes insulin resistance (Park K.S. et al 1999).

Streptozotocin causes oxidant mediated repression of mitochondrial transcription (Kristal B.S. et al 1997) and the quantity of mitochondrial DNA decreases in the islets of diabetes prone GK rats (Serradas P. et al 1995). Forty two different mitochondrial DNA point mutations, deletions and substitutions have been associated with NIDDM (Matthews C.E. et al 1998). Mitochondrial DNA mutations such as the M3243 base substitution can also cause maturity onset diabetes of the young (MODY) and auto antibody positive insulin dependent diabetes mellitus (IDDM) (Oka Y. 1993 and 1994). Free radicals can cause deletions of the mitochondrial genome (Wei Y.H. et al 1996). Nitric oxide and hydroxyl radical production in response to environmental agents were proposed as a means of producing mitochondrial DNA damage, expression of mutated proteins which cause MHC restricted immune responses and β cell death in Type 1 diabetes by Gerbitz K.D. (1992). Reductions in β cell numbers and islet amyloidosis containing islet amyloid polypeptide occurs in a high percentage of NIDDM patients (Clark A. et al 1995).

These defects impair oxidative phosphorylation, such impairment diminishing insulin secretion. Treatment with coenzyme Q10 has been reported to be successful in a patient with the M3243 A to G mutation (Suzuki Y. et al 1995). Glucagon secretion is also decreased in diabetes mellitus associated with mitochondrial DNA defects (Odawara M. et al 1996).

Insulin dependent diabetes, autoantibody positive also occurs in patients carrying the M3243 mutation. (Oka Y. et al 1993). 8-hydroxydeoxyguanosine (8OHdG) content and extent of deletion of mitochondrial DNA base 4977 deletion correlates with duration of NIDDM and the frequency of diabetic proliferative and simple retinopathy and nephropathy (Suzuki Y. et al 1999). Hyperglycemia causes oxidative damage to the mitochondrial DNA of

vascular smooth muscle and endothelial cells precipitating vasculopathy (Fukagawa N.K. et al 1999). High insulin levels are also implicated in damaging smooth muscle and endothelial cells (O'Brien S.F. et al 1997). Monosaturated palmitic acid causes DNA fragmentation of rat islet cells in culture. It also reduces the β cell proliferation caused by hyperglycemia. Palmitic acid also induced release of cytochrome c and apoptosis of β cells (Maedler K. et al 2001).

The methyl ester of succinic acid may bypass defects in glucose transport, phosphorylation and further catabolism and stimulate insulin secretion and release (McDonald J. et al 1988 and Malaisse W.J. et al 1994). Succinate esters increase the supply of succinic acid and acetyl CoA to the Krebs cycle (Malaisse W.J. 1993a), they stimulate insulin synthesis and release (Malaisse W.J. et al 1993b), they increase insulin output at high concentrations of glucose (Akkan A.G. et al 1993), they maintain insulin secretion when β cells are challenged with streptozotocin (Malaisse W.J. 1994), they enhance the insulinotropic effect of hypoglycemic sulfonylureas (Vicent D. et al 1994), they improve the secretory potential of exocrine pancreas when administered prior to streptozotocin (Akkan A.G. et al 1993), they protect against the cytotoxic effect of interleukin-1 (Eizirik D.L. et al 1994) and they do not show any glucagonotropic effect (Vicent D. et al 1994).

Glutamate also stimulates exocytosis of insulin, primarily by an intracellular mechanism acting downstream of mitochondrial metabolism, as oligomycin that abolishes the insulin release response to succinate does not inhibit the insulin release caused by glutamate (Maechler P. et al 2000). Also glutamate induced insulin release seems to require other factors such as ATP induced closure of potassium channels followed by influx of calcium and exocytosis.

Hyperglycemia increases the activity of protein kinase C (Lee T.S. et al 1989). Activation of protein kinase C increases the trans endothelial permeability of proteins such as

albumin (Lynch J.J. et al 1990). Albumin, hyperglycemia, H₂O₂ can cause the 4977 bp mitochondrial DNA deletion associated with diabetes (Egawhary, D.N. et al 1995 and Swoboda, B.E. et al 1995). Circulating endothelial cells containing this deletion are particularly common in patients with nephropathy and peripheral vascular disease.

5 The same deletion is also present during aging and more frequently in patients with impaired glucose tolerance or insulin resistance, hyperglycemia and free radicals being precipitants thereof (Liang P. et al 1997).

Taurine (Trachtman H. et al 1995) and vitamin C (Craven P.A. et al 1997) reduced glomerular hypertrophy, albuminuria, glomerular collagen and TGF- β 1 accumulation in a streptozotocin induced diabetic rat model.

10 In streptozotocin induced diabetes metal distribution is altered, there are increases in the quantities of hepatic copper, zinc, manganese, renal copper and zinc and plasma zinc. Insulin administration returns the metal levels to within normal ranges (Failla M.I. et al 1981). In contrast to the elevated hepatic and renal zinc concentrations in diabetic pregnant rats, their
15 fetuses have lower concentrations of hepatic zinc Uriu-Hare J. et al 1988). Higher groundwater zinc concentrations reduces the incidence of insulin dependent diabetes mellitus in childhood (Haglund B. et al 1996). Low serum zinc and hyperzincuria have been reported in the initial stages of Type 1 diabetes (Hagglof B. et al 1983). Hyperzincuria and borderline zinc deficiency also occurs in type II diabetes (Kinlaw W.B. et al 1983). Preloading animals
20 with zinc, which induces metallothionein synthesis, metallothionein being a radical scavenger, partially prevents streptozotocin induced diabetes (Yang Y. et al 1994). Elevated metallothionein increased resistance to DNA damage and to depletion of NAD⁺, increased resistance to hyperglycemia and reduced β cell degranulation and necrosis (Chen H. et al
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2001). Metallothionein is highly inducible and does not seem to have deleterious effects at higher concentrations.

In alloxan induced diabetes diethylenetriamine pentaacetic acid inhibits the hyperglycemic response (Grankvist K. et al 1983). Part of the cytoprotective effect of spirohydantoin-derivative aldose reductase inhibitors in diabetes may relate to their ability to chelate copper ions and thus inhibit ascorbic acid oxidation (Jiang Z.Y. et al 1991).

Iron-catalyzed peroxidative reactions may account for the diabetes found as a common side effect of transfusion siderosis, dietary iron overload and idiopathic hemochromatosis (McLaren G.D. et al 1983). Plasma copper levels are higher in diabetic patients and are highest in diabetics with angiopathy and diabetics who have alterations in lipid metabolism (Mateo M.C.M. et al 1978, Noto R. et al 1983). Carboxymethyl lysine (CML) levels are twice as high in the skin collagen of diabetics as compared with age matched controls (Dyer G.D. et al), and correlate positively with the presence of retinopathy and nephropathy (McCance D.R. et al 1993).

Matrix metalloproteinase-9 (MMP-9) concentrations are increased in noninsulin dependent diabetes mellitus (NIDDM) prior to development of microalbuminuria (Ebihara I. et al 1998). This proteinase is activated by zinc, calcium and oxidative stress.

Treatment with antioxidants polyethylene glycol-superoxide dismutase and N-acetyl-L-cysteine reduces MMP-9 activity (Uemura S. et al 2001). Increased MMP-9 activity is also observed in myocardial infarction, unstable angina and in atherosclerosis.

Polyamines as chelates of redox metals can prevent the metal and oxidative damage caused by metal overload, redistribute metals to storage sites and induce metallothionein.

Vanadium decrease blood glucose and D-3-hydroxybutyrate levels in diabetes, it also restores fluid intake and body weight of diabetic animals.

These metabolic effects occur because vanadium decreases P-enolpyruvate carboxykinase (PEPCK) transcription, thus decreasing gluconeogenesis; secondly it decreases tyrosine aminotransferase gene expression, Thirdly it increases expression of glucokinase gene; fourthly it induces pyruvate kinase; fifthly it decreases mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (HMGCoAS) gene expression; sixth it decreases the expression of the liver and pancreas glucose-transporter GLUT-2 gene in diabetic animals to the level seen in controls (Valera A. et al 2001); seventh it increases the amount of the insulin-sensitive glucose transporter, GLUT4 by stimulating its transcription (Strout H.V. et al); eighth the metabolic effects of vanadium are mediated by inhibition of protein tyrosine phosphatases (PTP). Peroxovanadium compounds irreversibly oxidize the thiol group of the essential cysteine at the PTP catalytic site (Fantus I.G. et al 1998). Vanadium is a structural analog of phosphate. Vanadium does not exhibit the growth effects and mitogenic effects of insulin and thus might avoid the macrovascular diseases consequences of hyperinsulinemia and be clinically useful in disease where insulin resistance is caused by defects in the insulin signaling pathway. Vanadium mimics the effects of insulin in restoring G proteins and adenyl cyclase activity increasing cyclic AMP levels. (Anand-Srivastava M.B. et al 1995); ninth vanadyl ion suppresses nitric oxide production by macrophages (Tsuji A. et al 1996); tenth it has a positive cardiac inotropic effect (Heyliger C.E. et al 1985); eleventh vanadium restores albumin mRNA levels in diabetic animals by increasing hepatic nuclear factor 1 (HNF 1) (Barrera Hernandez G. et al 1998); twelfth it restores triiodothyronine T₃ levels (Moustaid N. et al 1991).

In type I diabetes vanadium appears to reverse defects secondary to chronic insulin deficiency and hyperglycemia and may be useful in newly diagnosed diabetics who still have pancreatic reserve (Cam M.C. et al 2000). Vanadium is also β cell protective in streptozotocin diabetic rats (Cam M.C. et al 1999). In type II diabetes vanadium improves glucose tolerance

whilst decreasing plasma insulin levels. Improvement occurs in fasting plasma glucose, glycosylated hemoglobin levels, insulin stimulated glucose uptake and reduction of hepatic glucose output (Cohen N. et al 1995). Free fatty acid and triglyceride levels are controlled more quickly in diabetic animals than glucose levels (Cam M.C. et al 1993). Type I and Type II diabetic patients treated with vanadium had significantly less need for insulin (Goldfine A.B. et al 1995 & 2000).

The toxicity of vanadate was reduced by administering it in chelate form, sodium 4,5 dihydroxybenzene-1,3 disulfonate (Tiron) (Domingo J.L. et al 1995). The organic forms of vanadium corrected the hyperglycemia and impaired hepatic glycolysis more safely and potentially than vanadium sulphate (Reul B.A. et al 1999).

As with low zinc consumption predisposing to IDDM, dietary chromium deficiency has been associated with development of atherosclerosis and glucose intolerance. Chromium concentration in human tissues decreases very considerably after the first two decades of life. Further chromium excretion by the kidney is increased following oral glucose loading (Schroeder H.A. 1967). Modern diets containing refined carbohydrates have been depleted of their chromium content. Chromium concentrations in the hair of insulin dependent diabetic children were significantly lower than in controls (Hambidge K.M. et al 1968). Hepatic chromium concentrations were significantly decreased in diabetics and non significantly in atherosclerotic patients (Morgan J.M. 1972). Patients who died of cardiovascular diseases had lower aortic chromium concentrations than controls (Schroeder H.A. et al 1970). Human subjects with impaired glucose tolerance had significant improvement in impaired glucose tolerance, reduction of the exaggerated insulin response to a glucose load and reduction of serum cholesterol in response to chromium (Freiberg J.M. et al (1975). In spontaneously hypertensive rats chromium lead to a significant reduction in plasma glucose without

significant effect on plasma insulin following intraperitoneal glucose challenge (Yoshimoto S. et al 1992). Chromium supplementation in diabetics improves glucose tolerance, decreases blood cholesterol and triglycerides, and increases high density lipoprotein (HDL) (Abraham A.S. et al 1992).

5 Plasma chromium levels and insulin levels after oral glucose loading were higher in obese controls than in lean controls, plasma chromium levels were similar in obese and lean insulin dependent diabetics (IDD), plasma chromium levels were higher in lean non insulin dependent diabetics (NIDD) than in controls. Chromium levels correlate with body mass index (BMI) and rise in the obese and in non insulin dependent diabetics (NIDD) in response to insulin resistance. Chromium excretion was significantly increased in lean insulin dependent diabetics (IDD) (Earle K.E. et al 1989).

Atherosclerosis

15 In the hearts of patients having coronary artery disease the levels of mitochondrial DNA deletions M4977, M7436, M10,422 increase significantly and especially in left ventricle muscle, this area accumulating twenty seven times as many deletions as the left atrium (Corral-Debrinski M et al 1992). Ischemia causes a decrease in reduced glutathione and superoxide dismutase activity in heart (Ferrari R. et al 1985). Hearts that have experienced acute myocardial infarction have elevated levels of mitochondrial DNA over controls though lesser elevation than occurs in coronary artery disease hearts (Ferrari R. et al 1996). Reduced pH and increase Pi result from accumulation of lactate and hydrolysis of ATP. Reduced pH and increased Pi downregulate contractility and cause akinesia of the ischemic zone. GF-

109293X protects against hypoxia induced apoptosis in cardiac myocytes (Chen S.J. et al 1998).

The severity of clinical symptoms and survival time correlated with mitochondrial DNA defects in cardiomyopathy patients and hundreds of different DNA minicircles were observed (Ozawa T. et al 1995). Decreased activity levels of Complex I, III, IV and V occur in cardiomyopathy patients inheriting mutations or deletions of mitochondrial DNA (Marin-Garcia J. et al 1999) and depletion of mitochondrial DNA (Marin-Garcia J. et al 1988). Fifty percent of patients with hypertrophic cardiomyopathy were observed to have respiratory chain abnormalities (Zeviani M. et al 1995). Alcohol, ischemia and adriamycin also cause cardiomyopathy with mitochondrial DNA deletions. Mitochondrial DNA defects occur less frequently in dilated cardiomyopathy as compared with hypertrophic cardiomyopathy (Arbustini E. 1998 and 2000). Coenzyme Q₁₀ has been found to be an effective therapy in cardiomyopathy and in the treatment of congestive heart failure (Langsjoen P.H. et al 1988).

Stroke

Decreased levels of ATP, low pH, increase levels of intracellular glutamate, intracellular calcium ions and free radicals and protein kinase C activity occur during and post stroke. DNA fragmentation and oxidative damage occur (Chen J. et al 1997 and Cui J. et al 2000). Mitochondrial damage and cell death cause release of large quantities of redox metals locally in the area of the lesion. Endoplasmic reticulum releases calcium and this can be prevented in experimental stroke by dantrolene (Tasker R.C. et al 1998) Uric acid, a scavenger of peroxynitrite and hydroxyl radicals (Yu F. et al 1998), vitamin E (Tagami M. et al 1999) and estrogen (Goodman Y. et al 1996) can prevent apoptosis in stroke models.

Presbycusis

Presbycusis results from mitochondrial DNA mutations such as the M3243 point mutation (Bonte C.A. et al 1997). Acetyl-L-carnitine and α -lipoic acid protected rats from developing hearing loss and diminished the quantity of mitochondrial DNA deletions which accumulated during aging (Seidman M.D. et al 2000). These compounds can be effective in upregulating cochlear mitochondrial function.

Cancer

Cell Division / Growth Factors

During the synthesis phase of cell division methionine is increasingly converted to homocysteine thiolactone, thioethionine is converted to thiocysteine and cobalamin is removed from binding to mitochondrial and endoplasmic reticulum membranes. Thus increased quantities of oxygen radical species are produced. Homocysteine is formed by oxidation of homocysteine thiolactone (McCully K.S 1971). Homocysteine stimulates release of growth factors such as insulin like growth factor (Clopach P. et al 1976).

Depletion of thioethionine in aging and cancer

Depletion of thioethionine from mitochondrial and microsomal membranes causes increased formation of oxygen radicals and their release within neoplastic and senescent cells (Olszewski A.J. et al 1993). Depletion of thioethionine from mitochondrial and microsomal membranes causes; excessive homocysteine thiolactone synthesis; increased conversion of thioethionine to thiocysteine; inhibition of oxidative phosphorylation; and accumulation of toxic

oxygen radical species McCully 1994a). Malignant cells accumulate homocysteine thiolactone. Deficient intracellular methionine and adenosyl methionine in malignant cells may result from excessive conversion of methionine to homocysteine lactone.

Metabolites and retinoic acid

Folic acid and riboflavin are required for the conversion of homocysteine to methionine. Reduced folate intake is associated with increased incidence of heart disease and stroke. Also DNA damage from hypomethylation occurs due to deficiency of adenosyl methionine.

Pro carcinogenic and anti carcinogenic compounds

Thioretinaco and thioretinamide are cytostatic in cultured malignant cells (McCully K.S. 1992). Homocysteine thiolactone causes fibrosis, necrosis, inflammation, squamous metaplasia, dysplasia, neoplasia, calcification and angiogenesis (McCully K.S et al 1989, 1994a). Homocysteine induces apoptosis (Kruman I. et al 2000). Secondary increase in homocysteine thiolactone leads to disulphide bond formation with amino acids. Homocysteic acid is produced by from oxidation of homocysteine thiolactone.

Neovascularization

Oxygen radicals cause tissue damage during neovascularization. Arteriosclerosis is observed in the new vasculature as cancer grows and invades. Atherogenesis is correlated with total homocysteine. Homocysteine is correlated with total cholesterol and low density lipoprotein (LDL) + high density lipoprotein (HDL) cholesterol McCully K.S. 1990) Increased synthesis of homocysteine thiolactone enhances atherogenesis because of thiolation of amino

acids of apoB of low density lipoprotein producing aggregation and uptake of LDL by macrophages.

ATP formation and oxygen species holding

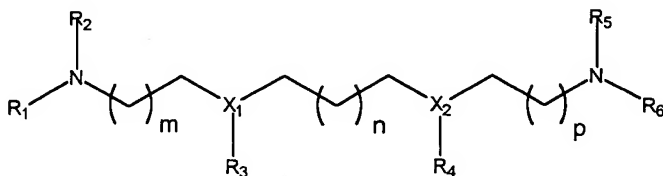
Under normal circumstances the disulfonium form of thioretinaco, in the presence of ascorbate, is the electrophile that catalyzes reduction of radical oxygen species to water, concomitant with binding of ATP from the F1 complex 1994a,b). Binding of the oxygen anions of the proximal and terminal phosphates of ATP to the disulfonium complex releases ATP from the F1 binding site McCully K.S. 1994a). Adenosyl methionine formation and further formation of thioretinaco result from cleavage of the adenosyl triphosphate bond.

SUMMARY OF THE INVENTION

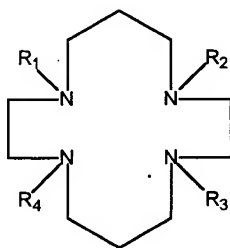
The invention is a process for synthesizing polyamine compounds via a series of substitution reactions, optimizing the bioavailability and biological activities of the compounds, and their use as therapeutic agents for the treatment of Parkinson's disease, Alzheimer's disease, Lou Gehrig's disease, Binswanger's disease, Olivopontine Cerebellar Degeneration, Lewy Body disease, Diabetes, Stroke, Atherosclerosis, Myocardial Ischemia, Cardiomyopathy, Nephropathy, Ischemia, Glaucoma, Presbycusis and Cancer. Tetraamines and polyamines produced herein are compounds that act as bases and which can be prepared by the reaction of acyclic and cyclic amines or alkyl halides with a variety of substrates that will add to the amines or displace the halides. These tetraamines fall into a number of structural classes. These classes are: (1) predominately linear tetraamines and polyamines linked by 1,3-

propylene and/or ethylene groups; (2) predominately branched tetraamines and polyamines linked by 1,3-propylene and/or ethylene groups; (3) cyclic polyamines linked by 1,3-propylene and/or ethylene groups; (4) combinations of linear, branched and cyclic polyamines linked by one or more 1,3-propylene and/or ethylene groups, (5) substituted polyamines. Further, the linked tetraamines may have one or more pendant alkyl, aryl cycloalkyl or heterocyclic moieties attached to the nitrogens.

Accordingly, in one aspect the invention is directed to compounds of the formula:



or



Wherein

R₁ and R₂ may be the same or different and are hydrogen, alkyl, aryl, cycloalkyl, amino acid, glutathione, uric acid, ascorbic acid, taurine, estrogen, dehydroepiandrosterone, probucol, vitamin E, hydroxytoluene, carvidilol, α-lipoic acid, α-tocopherol, ubiquinone,

phyloquinone, β -carotene, meanadione, glutamate, succinate, acetyl-L-carnitine, co-enzyme Q, lazeroids, polyphenolic flavonoids, homocysteine, menaquinone, idebenone, dantrolene, -
 $(\text{CH}_2)_n[\text{XCH}_2]_n\text{NH}_2$ - wherein $n = 3-6$ and $X =$ nitrogen, sulfur, phosphorous or carbon, or
heterocycle wherein R_1 and R_2 taken together are $-(\text{CH}_2\text{XCH}_2)_n-$ wherein $n = 3-6$ and $X =$

R_3 and R_4 may be the same or different and are hydrogen, alkyl, aryl, cycloalkyl, amino acid, glutathione, uric acid, ascorbic acid, taurine, estrogen, dehydroepiandrosterone, probucol, vitamin E, hydroxytoluene, carvidilol, α -lipoic acid, α -tocopherol, ubiquinone, phyloquinone, β -carotene, meanadione, glutamate, succinate, acetyl-L-carnitine, co-enzyme Q, lazeroids, polyphenolic flavonoids, homocysteine, menaquinone, idebenone, dantrolene or heterocycle wherein R_3 and R_4 taken together are $-(\text{CH}_2\text{XCH}_2)_n-$ wherein $n = 3-6$ and $X =$ nitrogen, sulfur, phosphorous or carbon.

R_5 and R_6 may be the same or different and are hydrogen, alkyl, aryl, cycloalkyl, amino acid, glutathione, uric acid, ascorbic acid, taurine, estrogen, dehydroepiandrosterone, probucol, vitamin E, hydroxytoluene, carvidilol, α -lipoic acid, α -tocopherol, ubiquinone, phyloquinone, β -carotene, meanadione, glutamate, succinate, acetyl-L-carnitine, co-enzyme Q, lazeroids, polyphenolic flavonoids, homocysteine, menaquinone, idebenone, dantrolene -
 $(\text{CH}_2)_n[\text{XCH}_2]_n\text{NH}_2$ - wherein $n = 3-6$ and $X =$ nitrogen, sulfur, phosphorous or carbon, or heterocycle wherein R_5 and R_6 taken together are $-(\text{CH}_2\text{XCH}_2)_n-$ wherein $n = 3-6$ and $X =$ nitrogen, sulfur, phosphorous or carbon.

M , n , and p may be the same or different and are bridging groups of variable length from 3-12 carbons.

X_1 and X_2 may be the same or different and are nitrogen, sulfur, phosphorous or carbon.

As used herein, "alkyl" has its conventional meaning as a straight chain or branched chain saturated hydrocarbyl residue such as methyl, ethyl, propyl, isopropyl, isobutyl, t-butyl, octyl, decyl and the like. The alkyl substituents of the invention are of 1 to 12 carbons which may be substituted with 1 to 2 substituents.

"Cycloalkyl" refers to a cyclic alkyl structure containing 3 to 25 carbon atoms. The cyclic structure may have alkyl substituents at any position. Representative groups include cyclopropyl, cyclopentyl, cyclohexyl, 4-methylcyclohexyl, cyclooctyl and the like.

"Aryl" refers to aromatic ring systems such as phenyl, naphthyl, pyridyl, quinolyl, indolyl and the like; aryl alkyl refers to aryl residues linked to the position indicated through an alkyl residue.

"Heterocycle" refers to ringed moieties with rings of 3-12 atoms and which contain nitrogen, sulfur, phosphorus or oxygen.

As shown from the above structures, examples include derivatives of 1,3-bis-[(2'-aminoethyl)-amino]propane (referred to hereafter as 2,3,2-tetramine); 1,4-bis-[(3'-aminopropyl)-amino]butane (referred to as 3,3,3-tetramine); and 1,4,8,11-Tetraazacyclotetradecane (cyclam). Specific examples include N,N',N'',N'''-tetramethyl 2,3,2-tetramine; N,N'''-dimethyl 2,3,2-tetramine; N,N'''-Dipiperidyl-2,3,2-tetramine, N,N',N'',N'''-tetramethylcyclam and N,N',N'',N'''-tetraadamantylcyclam.

Particularly preferred embodiments of R₁ and R₄ are piperidine, piperazine, or adamantane. In this embodiment, N₁ and N₄ are part of the piperidine or piperazine rings while in the adamantane case, N₁ and N₄ are appended from the rings.

It will be understood that the compounds of 1 and 2, since they contain a basic amine group, form salts with non-toxic acids and such salts are included within the scope of this invention. These salts may enhance the pharmaceutical application of the compounds.

Representative of such salts are the hydrochloride, hydrobromide, sulfate, phosphate, acetate, lactate, glutamate, succinate, propionate, tartrate, salicylate, citrate and bicarbonate.

There are two structural motifs that are being exploited in this invention. 1,3-bis-[(2'-aminoethyl)-amino]propane (2,3,2-tetramine) and its derivatives are tetramines that are known to have a large number of physiological actions. They are well known binders of metal ions and form very stable complexes with a variety of transition metals. Secondly, polyazamacrocycles such as 1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane (cyclam) are of considerable interest due to their ability to form strong complexes with transition metals such as copper, cobalt, iron, zinc, cadmium, manganese and chromium.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1-18 depict reaction schemes for the preparation of a variety of intermediates and the subsequent polyamines described in the invention as follows:

Figure 1 Route of Synthesis of 1,3-bis-[(2'-aminoethyl)-amino]propane and analogous compounds

Figure 2 Route of Synthesis of [2-(methylethylamino)ethyl](3-{[2-(methylamino)ethyl]amino}propyl)amine and analogous compounds

- Figure 3 Route of Synthesis of (2-piperidylethyl)-{3-[(2-piperidylethyl)amino]propyl}amine and analogous compounds
- Figure 4 Route of Synthesis of (2-piperaziny lethyl)-{3-[(2-piperaziny lethyl)amino]propyl}amine and analogous compounds
- Figure 5 (2-aminoethyl){3-[(2-aminoethyl)methylamino]propyl}methylamine and analogous compounds
- Figure 6 [2-(bicyclo[3.3.1]non-3-ylamino)ethyl](3-{2-(bicyclo[3.3.1]non-3-ylamino)ethyl}amino)propyl}amine and analogous compounds
- Figure 7 (2-aminoethyl){3-[(2-aminoethyl)amino]-1-methylbutyl}amine and analogous compounds
- Figure 8 (2-pyridylmethyl){3-[(2-pyridylmethyl)amino]propyl}amine and analogous compounds
- Figure 9 methyl(3-[methyl(2-pyridylmethyl)amino]propyl)(2-pyridylmethyl)amine and analogous compounds
- Figure 10 [2-(dimethylamino)ethyl](3-{[2-(dimethylamino)ethyl]methylamino}propyl)methylamine and analogous compounds

Figure 11 2-[3-(2-aminoethylthio)propylthio]ethylamine and analogous compounds

Figure 12 1,4,8,11-tetraaza-1,4,8,11-tetramethylcyclotetradecane and analogous compounds

Figure 13 1,4,8,11-tetraaza-1,4,8,11-tetra(2-piperidylethyl)cyclotetradecane and analogous compounds

Figure 14 1,4,8,11-tetraaza-1,4,8,11-tetrabicyclo[3.3.1]non-3-ylcyclotetradecane and analogous compounds

Figure 15 1,4,8,11-tetraaza-1,4,8,11-tetraethylcyclotetradecane and analogous compounds

Figure 16 N,N'-(2'-dimethylphosphinoethyl)-propylenediamine

Figure 17 Vanadyl 2,3,2-Tetramine

Figure 18 Chromium 2,3,2-Tetramine

Figure 19 Schematic of 2,3,2, tetramine structure; 1,3-bis-[(2'-aminoethyl)-amino]propane

DESCRIPTION OF PREFERRED EMBODIMENTS

HEATS OF FORMATION

5 Among the reasons for the selection of compounds to be used for these formulations are the results of a series of calculations using heats of formations of the molecules. The relative stabilities of the compounds were determined in order to predict which would lead to the most stable metal complexes when they react with metals such as copper, cobalt, iron, zinc, cadmium, manganese and chromium. These metals are of particular interest due to their importance in neurological and other diseases.

10 Heats of formation (ΔH°) are calculated by looking at the formation of a compound from its constituent atoms. The lower the heat of formation, the more stable is the compound. The assumption in this computational work is that the calculated heats of formation for the complexes will correlate with the ability of the organic compound to complex with metal ions in biological systems. The more strongly the binding occurs, the more likely it is that the organic molecule will interact with the metal ion of choice. There are other factors that enter into the actual binding ability of the organic molecules, but heats of formation help suggest how different organic molecules might behave. By varying the organic molecules, the heats of formation for the complexes can be compared and correlations between the stability of the complexes and the structure of the complexes can be made. The relative stabilities of a representative survey of organic compounds is shown in Table I while the heats of formation for the metal complexes are shown in Tables II-VIII.

Table I. Heats of Formation of Organic Compounds

Compound	ΔH° (Kcal/mol)
2,3,2-tetramine	-18.24
2,2,2-tetramine	-17.09
3,3,3-tetramine	-32.70
2,3,2-methylated on N1/N4	-13.81
2,3,2-methylated on N2/N3	-10.35
2,3,2-piperidine	-32.47
2,3,2-piperizine	4.33
2,3,2-tetra sulfur	-26.25
cyclam	-15.65
cyclam-methylated	18.73
cyclam-adamantane	-40.02

Table II. Heats of Formation of Copper Complexes

Compound	ΔH° (Kcal/mol)
Cu 2,3,2-tetramine	244.10
Cu 2,2,2-tetramine	252.36
Cu 3,3,3-tetramine	224.16
Cu 2,3,2-methylated on N1/N4	243.98
Cu 2,3,2-methylated on N2/N3	241.42
Cu 2,3,2-isopropyl on N1/N4	207.69
Cu 2,3,2-isopropyl on N2/N3	250.17
Cu 2,3,2-dibenzyl on N2/N3	314.08
Cu 2,3,2-tetramethyl	273.85
Cu 2,3,2-tetraisopropyl	229.83
Cu 2,3,2-benzylated	380.10
Cu 2,3,2-piperidine	255.10
Cu 2,3,2-piperizine	288.68
Cu 2,3,2-adamantane	269.53
Cu 2,3,2-methyls on carbons 5/7	227.45
Cu 2,3,2-tetra sulfur	210.42
Cu cyclam	260.20
Cu cyclam-methylated	298.97
Cu cyclam-benzylated	405.60
Cu cyclam-adamantane	254.55
Cu cyclam-isopropyl	271.59
Cu cyclam-S4	207.15
Cu cyclen	285.10
Cu cyclam 3,3,3	245.28

Table III. Heats of Formation of Iron Complexes

<i>Compound</i>	ΔH° (Kcal/mol)
Fe 2,3,2	12.16
Fe 2,2,2	37.16
Fe 3,3,3	-1.39
Fe 2,3,2-methylated on N1/N4	-8.19
Fe 2,3,2-piperidine	-54.23
Fe 2,3,2-piperizine	-18.51
Fe 2,3,2-adamantane	-19.16
Fe 2,3,2-methyls on carbons 5/7	7.99
Fe 2,3,2-tetra sulfur	87.39
Fe cyclam	-5.75
Fe cyclam-methylated	-69.53
Fe cyclam-adamantane	-92.82
Fe cyclam-isopropyl	-83.02
Fe cyclam-S4	137.13
Fe cyclen	17.76
Fe cyclam 3,3,3	-31.73

Table IV. Heats of Formation of Zinc Complexes

<i>Compound</i>	ΔH° (Kcal/mol)
Zn 2,3,2	355.75
Zn 2,2,2	352.45
Zn 3,3,3	328.73
Zn 2,3,2-methylated on N1/N4	336.55
Zn 2,3,2-isopropyl on N1/N4	316.18
Zn 2,3,2-isopropyl on N2/N3	330.81
Zn 2,3,2-tetramethyl	351.00
Zn 2,3,2-benzylated	478.96
Zn 2,3,2-piperizine	351.70
Zn 2,3,2-methyls on carbons 5/7	342.21
Zn 2,3,2-tetra sulfur	329.15
Zn cyclam	358.25
Zn cyclam-methylated	388.64
Zn cyclam-benzylated	485.39
Zn cyclam-adamantane	347.52
Zn cyclam-isopropyl	330.81
Zn cyclam -S4	339.04
Zn cyclam 3,3,3	351.89

Table V. Heats of Formation of Manganese Complexes

Compound	ΔH° (Kcal/mol)
Mn 2,3,2	266.79
Mn 2,2,2	235.44
Mn 3,3,3	194.42
Mn 2,3,2-tetra sulfur	264.50
Mn cyclam	215.97
Mn cyclam-methylated	198.40
Mn cyclam -S4	248.57

Table VI. Heats of Formation of Cobalt Complexes

Compound	ΔH° (Kcal/mol)
Co 2,3,2	-1250.81
Co 2,2,2	-1236.41
Co 3,3,3	-1265.92
Co 2,3,2-methylated on N1/N4	-1269.13
Co 2,3,2-piperidine	-1300.69
Co 2,3,2-adamantane	-1250.92
Co 2,3,2-methyls on carbons 5/7	-1268.45
Co 2,3,2-tetra sulfur	-1258.52
Co cyclam	-1187.9
Co cyclam-methylated	-1265.64
Co cyclam-isopropyl	
Co cyclam -S4	-1265.56

Table VII. Heats of Formation of Cadmium Complexes

Compound	ΔH° (Kcal/mol)
Cd 2,3,2	393.21
Cd 2,2,2	401.00
Cd 3,3,3	382.04
Cd 2,3,2-isopropyl on N1/N4	366.86
Cd 2,3,2-isopropyl on N2/N3	376.40
Cd 2,3,2-piperidine	374.06
Cd 2,3,2-adamantane	354.51
Cd 2,3,2-tetra sulfur	357.79
Cd cyclam	411.95
Cd cyclam-isopropyl	376.40
Cd cyclam-S4	356.13

1 **Table VIII. Heats of Formation of Chromium Complexes**

2

3 *Compound* *ΔH° (Kcal/mol)*

4

5 Cr 2,3,2 398.73

6 Cr 2,3,2-isopropyl on N1/N4 379.87

7 Cr 2,3,2-piperidine 403.22

8 Cr cyclam 399.99

9 Cr cyclam-isopropyl 430.05

10 This tabular data can be analyzed by comparing the various structural features of the

11 molecules as in examples 19 to 24 below.

12

13

14 **PREPARATION OF THE INVENTION COMPOUNDS**

15

16 There are numerous compounds described in the invention but in general, the invention

17 compounds are obtained by converting the starting di- or tetramine of the formula:

18

19 A variety of reactions were used to prepare the compounds. Compound 1 was prepared

20 via a nucleophilic substitution reaction followed by conversion of the free amine to its HCl

21 salt. The amine acts as the nucleophile in displacing the di-alkyl halide, a reaction of general

22 utility. Compound 2 also involved a nucleophilic substitution reaction, this time done in basic

23 solution with a protection/deprotection sequence also involved in the synthesis. The use of

24 acetyl groups to protect the amines could be exploited to alkylate tetramines.

25 Compounds 3 and 4 were synthesized by having 1,3-diaminopropane serve as the

26 nucleophile with displacement occurring on the α carbon to the piperidine or piperazine. The

27 β position is particularly susceptible to nucleophilic attack in molecules of this type. Other

28

heterocyclic moieties could be added in similar fashion starting from the appropriate β -ethyl heterocycle in this fashion.

The theme of using amines to attack alkyl halides in nucleophilic substitution reactions was also exploited in the formation of **6** and **14**. The 1-position in the bromoadamantane is much more reactive than expected and so the adamantane moiety could be added to numerous amines in this fashion. Compound **7** involves a novel preparation of an existing compound as we reversed the nature of the nucleophile and the electrophile to lead to high yields of the product. In the case described, the 1,3-substituted portion is the alkyl halide while the amine is used to form the terminal nitrogens.

Compounds **8** and **9** were prepared using substitution reactions rather than the previously reported (for **8**) imine formation reaction followed by a reduction. The α -carbon on the pyridine ring is extremely reactive due to resonance stabilization of any intermediate formed. This is a general approach and numerous other aromatic heterocycles could be added in this fashion.

We have continued to take advantage of nucleophilic substitution reactions to prepare **11** with the electrophilic 2-chloroethylamine. Once again, this scheme illustrates the extreme reactivity of the β -carbon on amines when used to do substitution reactions. 2-chloroethylamine could be added to many amines to form other tetraamines including many that are not symmetrical.

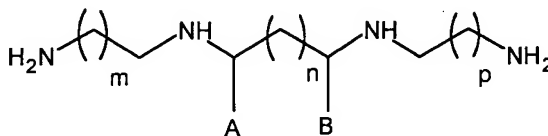
Compound **13** was prepared in a fashion similar to that used to synthesize **3**. The starting amine here is the macrocyclic cyclam. This reaction illustrates the power of using macrocycles in these schemes as the substitution led cleanly to the tetramine. Compound **15** was prepared under strongly basic conditions using the anion of the cyclam as the nucleophile attacking an alkyl halide. Certainly any primary alkyl halide could be substituted in this

sequence. Phosphine also can be incorporated into these molecules as been done for Compound 16. This molecule was prepared via the use of an addition/reduction sequence starting with an amine. This reaction could be used on any number of amines covered in this patent.

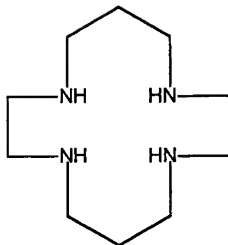
Compounds 1-16 can be used to make metal complexes. Examples include the preparation of the vanadium complex 17 where 2,3,2-tetramine is converted into its vanadium complex by treatment with a vanadium precursor. Compound 18 was prepared in similar fashion starting with a chromium precursor. Any number of metal complexes such as copper, cobalt, iron, manganese could be prepared from any of the compounds 1-16 by treating these compounds with the appropriate metal salt followed by isolation of the metal complex.

Compound 16 is a novel compound that incorporates phosphorous into the molecule in the place of two of the nitrogens. This internal substitution is done via an addition/reduction process and could be changed to include oxygen or other donors if desired.

The preparation of the vanadium(IV) complex 17 occurs in straightforward fashion by mixing a vanadium precursor with the 2,3,2-tetramine and isolating the complex. Compound 18 is prepared in similar fashion using a chromium precursor.



or



(where A and B equal hydrogen or alkyl and m, n, and p may be the same or different) to the corresponding N-substituted compound by treating these compounds with an alkyl halide under conditions that affect the conversion. There are also instances where some forms of 2,3,2-tetramine need to be protected prior to adding on the various groups as is true for 2 and 6. For the cyclam type molecules, nucleophilic substitution reactions were generally used to prepare the compounds (compounds 10-15).

Compounds 2, 3, 4, 6, 9, 13, and 14 are prepared in this invention for the first time. Of the known compounds described here, most (5, 7, 8, 10, 11, 12, and 15) have been prepared in a fashion significantly different than that found in the literature. In addition, many of the compounds covered in the invention but not used as examples have not been prepared elsewhere and will be prepared as part of this invention for the first time.

The base compound 1,3-bis-[(2'-aminoethyl)-amino]propane, 1, was prepared in a fashion similar to that found in the literature (Van Alphen, J. Rec. Trav. Chim. 55, 835, 1936). However, in the original literature preparation, an impurity was found that significantly reduced the purity of the product. Subsequent preparations have taken a number of tacks to lead to a pure product. We have eliminated this problem by developing a purification strategy that works through the hydrochloride salt that leads to a single product of very high purity.

In order to prepare the novel compound **2**, ([2-(methylethylamino)ethyl](3-{[2-(methylamino)ethyl]amino}propyl)amine), protection of the more reactive terminal nitrogens 1 and 4 as their acetyl derivatives was performed prior to methylation of nitrogens 2 and 3. Deprotection of the acetyl groups with KOH led to the desired compound.

Compounds **3** ((2-piperidylethyl)-{3-[(2-piperidylethyl)amino]propyl}amine) and **4** ((2-piperaziny lethyl)-{3-[(2-piperaziny lethyl)amino]propyl}amine) were made in similar fashion through the nucleophilic substitution reaction of 1,3-diaminopropane with 1-(2-chloroethyl)piperidine (to give **3**) or 1-(2-chloroethyl)piperazine (to give **4**). Numerous other tetramines are accessible through similar reactions where the nature of the amine is varied.

(2-aminoethyl){3-[(2-aminoethyl)methylamino]propyl}methylamine, **5**, is a known compound (Barefield, E.K., Wagner, F., Hodges, K.D., Inorg. Chem., 15, 1370-1377, 1976) but was prepared in a novel way here. The physical properties of our compound do not match those found in the literature but the NMR data in the literature in no way fits the structure of the compound while our NMR and mass spectral data are consistent with the formulation.

Compound **6**, [2-(bicyclo[3.3.1]non-3-ylamino)ethyl](3-{2-(bicyclo[3.3.1]non-3-ylamino)ethyl}amino}propyl)amine and compound **14**, 1,4,8,11-tetraaza-1,4,8,11-tetrabicyclo[3.3.1]non-3-ylcyclotetradecane were prepared in a similar way. Direct amination (Krumkalns, E.V., Pfeifer, W., Jour. Med. Chem., 11, 1103, 1968) of 1-bromoadamantane with the appropriate amine led to the pure products.

Compound **7**, (2-aminoethyl){3-[(2-aminoethyl)amino]-1-methylbutyl}amine, has been prepared previously through the reaction of N,N'-bis(chloroacetyl)-2,4-pentanediamine with methylamine (Mikukami, F., Bull. Chem. Soc., Jpn., 48, 1205-1212, 1975). We have prepared the compound in a completely different way by following a similar procedure as that used for compound **1**.

(2-pyridylmethyl){3-[(2-pyridylmethyl)amino]propyl}amine, **8**, is a known compound but was prepared by a completely different procedure than that found in the literature. Instead of making this compound via the two step process of a Schiff base condensation of pyridine-2-carboxaldehyde with 1,3-propanediamine followed by a reduction reaction (Fischer, H.R., Hodgson, D.J., Michelsen, K., Pedersen, E., *Inorg. Chim. Acta*, **88**, 143-150, 1984), we prepared it directly through a nucleophilic substitution of picolyl chloride with 1,3-propanediamine. This results in higher overall yields since we employ a one step process.

The preparation of the novel compound **9**, methyl(3-[methyl(2-pyridylmethyl)amino]propyl)(2-pyridylmethyl)amine, was performed in a fashion similar to that used to synthesize **8**. The product was of high purity and its analytical data matched the desired structure.

Compound **10**, [2-(dimethylamino)ethyl](3-{[2-(dimethylamino)ethyl]methylamino}propyl)methylamin, was prepared by the literature procedure (Golub, G., Cohen, H., Meyerstein, D., *J. Chem. Soc., Chem. Commun.*, 397-398, 1992.) and the synthesis resulted in a high yield of a pure product. Although the literature did not supply physical data for the compound, our results are consistent with the structure of the compound.

2-[3-(2-aminoethylthio)propylthio]ethylamine, **11**, is a known compound (Hay, R.W., Gidney, P.M., Lawrance, G.A., *J. Chem. Soc., Dalton*, 779-784, 1975) but was prepared by a novel procedure here. Nucleophilic substitution of 1,3-dimercaptopropane with 2-chloroethylamine resulted in formation of **11** that had physical properties similar to those reported.

The preparation of 1,4,8,11-tetraaza-1,4,8,11-tetramethylcyclotetradecane, **12**, was performed in a manner similar to that found in the literature (Barefield, K., Wagner, F., *Inorg. Chem.*, **12**, 2435-2436, 1973). The analytical data for this compound matches that found previously.

Compound 13, 1,4,8,11-tetraaza-1,4,8,11-tetra(2-piperidylethyl)cyclotetradecane, was prepared from cyclam through a nucleophilic substitution in a fashion similar to the one we use to prepare compound 4. Many other derivatives of cyclam could be prepared using this type of reaction.

1,4,8,11-tetraaza-1,4,8,11-tetraethylcyclotetradecane, 15, is a known compound (Oberholzer, M.R., Neuburger, M., Zehnder, M., Kaden, T.A., *Helv. Chim. Acta*, 78, 505, 1995) but was prepared here by a modified procedure using similar reagents but with different reactions conditions and purification steps.

Compound 16 is a novel compound that incorporates phosphorous into the molecule in the place of the two nitrogens. This internal substitution is done via addition/reduction process and could be changed to include oxygen or other donors if desired.

The preparation of the vanadium (IV) complex 17 occurs in straightforward fashion by mixing a vanadium precursor with the 2,3,2-tetramine and isolating the complex. Compound 18 is prepared in similar fashion using a chromium precursor.

Compounds 1 to 18 correspond with Figures 1 to 18 and Examples 1 to 18.

The following examples are intended to illustrate but not to limit the number of compounds within the scope of the invention.

Example 1

1,3-bis-[(2'-aminoethyl)-amino]propane [Figure 1].

A mixture of 15 g of 1,3-dibromopropane and 50 mL of absolute EtOH was added slowly to 25 g of 1,2-diaminoethane hydrate. The mixture immediately became warm. It was

then heated to 50 °C for 1 hour, 20 g of KCl added and the heating continued for 30 minutes. The mixture was filtered from the KBr and distilled at reduced pressure. The residue formed two layers that were separated. The top layer was distilled and the product had a b.p. of 115-116 °C, (1 mm). The compound was further purified by converting the free amine to its tetrahydrochloride salt by addition of 6M. HCl. The melting point of the salt was 278-283 °C. It was converted back to its free amine by treatment with NH₄OH. Mass spectral analysis showed a m/e = 160. ¹H NMR (CDCl₃): δ 1.26 (6H, s), 1.60 (2H, quin), 2.60 (4H, t), 2.71 (8H, t).

Example 2

[2-(methylethylamino)ethyl](3-{[2-(methylamino)ethyl]amino}propyl)amine [Figure 2].

A mixture of 0.37 g (0.0155 mol) of magnesium turnings, 5.0 g (0.031 mol) of 1,3-bis-[(2'-aminoethyl)-amino]propane, 50 mL of benzene and 3.76 g (0.047 mol) of acetyl chloride is heated under reflux for 2 h. The reaction mixture is cooled in an ice bath and the liquid portion is decanted into a separatory funnel. The residue in the flask is washed twice with 50 mL portions of ether, and the ethereal solution is poured over ice. The ether-water mixture is then added to the benzene solution in the separatory funnel and separated. The organic phase is washed once with 50 mL of 5% sodium bicarbonate and once with water and dried over CaCl₂. The solution is filtered and used without further purification.

A magnetically stirred mixture of 5.0 g (8.67 mmol) of the acetylated 2,3,2-tetramine prepared above and 2.0 g (80.7 mmol) of sodium hydride in 75 mL of N,N-dimethylformamide was heated at 60 °C under N₂ for 3 h. The resultant mixture was treated with 19.8 g (0.164 mol) of iodomethane and stirred at 50 °C. After 24 h at 50 °C, the reaction

was quenched by the addition of 95% EtOH. Volatiles were removed at reduced pressure and 50 mL of water was added to the residue. The product was extracted with three 50 mL portions of chloroform. The combined organic extracts were successively washed with water and NaCl, dried over anhydrous sodium sulfate, and concentrated to give 6.3 g of yellowish oil. The oil was purified by flash chromatography with 1:4 hexanes-ethyl acetate as the eluent to give acetylated [2-(methylethylamino)ethyl](3-{[2-(methylamino)ethyl]amino}propyl)amine as an oil.

A stirred solution of 3.0 g (4.54 mmol) of acetylated [2-(methylethylamino)ethyl](3-{[2-(methylamino)ethyl]amino}propyl)amine, 10.0 g (0.178 mol) of potassium hydroxide, 70 mL of methanol and 15 mL of water was heated under reflux for 24 h. The methanol was removed at reduced pressure and the product was extracted into 2 x 50 mL of ether. The combined extracts were washed with NaCl, dried over sodium sulfate and concentrated under vacuum. The crude mixture was purified by flash chromatography with 5:1 hexanes-ethyl acetate as the eluent. After evaporation of the solvents, 0.79 g (71%) of the product was obtained as a colorless oil. Mass spectral analysis showed a $m/e = 244$. ^1H NMR (CDCl_3): δ 1.03 (12 H, d), 1.26 (6H, s), 1.60 (2H, quin), 2.60 (4H, t), 2.71 (8H, t), 3.23 (2H, m).

Example 3

(2-piperidylethyl)-{3-[(2-piperidylethyl)amino]propyl}amine [Figure 3].

To a mixture of 0.5 g (6.75 mmol) of 1,3-diaminopropane and 50 mL of absolute EtOH was added 1.62 g (40.5 mmol) of NaOH. To this solution was added dropwise 2.48 g (13.45 mmol) of 1-(2-chloroethyl)piperidine in 50 mL of EtOH over 30 min. The solution was allowed to stir for 24 h. The solvent was evaporated and the residue was extracted with 2 x 50

mL of CH₂Cl₂, dried over Na₂SO₄, and evaporated to dryness. The compound was purified by converting it to its hydrochloride salt by addition of HCl. The melting point of the salt was > 300 °C. It was converted back to its free amine by treatment with NH₄OH. The resultant oil (1.04 g, 52%) was analyzed. Mass spectral analysis showed a m/e = 297 (M⁺ + 1). ¹H NMR (CDCl₃): δ 1.40-1.82 (14H, m), 2.40-2.58 (14H, quin), 2.60-2.72 (10H, m).

Example 4

(2-piperazinyloethyl)-{3-[(2-piperazinyloethyl)amino]propyl}amine [Figure 4].

To a mixture of 0.5 g (6.75 mmol) of 1,3-diaminopropane and 50 mL of absolute EtOH was added 1.62 g (40.5 mmol) of NaOH. To this solution was added dropwise 2.48 g (13.45 mmol) of 1-(2-chloroethyl)piperazine in 50 mL of EtOH over 30 min. The solution was allowed to stir for 24 h. The solvent was evaporated and the residue was extracted with 2 x 50 mL of CH₂Cl₂, dried over Na₂SO₄, and evaporated to dryness. The compound was purified by converting it to its hydrochloride salt by addition of HCl. The melting point of the salt was > 300 °C. It was converted back to its free amine by treatment with NH₄OH. The resultant oil (0.82 g, 41%) was analyzed. Mass spectral analysis showed a m/e = 299 (M⁺ + 1). ¹H NMR (CDCl₃): δ 1.40-1.82 (10H, m), 2.42-2.55 (14H, quin), 2.58-2.77 (10H, m).

Example 5

(2-aminoethyl){3-[(2-aminoethyl)methylamino]propyl}methylamine [Figure 5].

To a solution of 1.0 g (0.0128 mol) of N,N-dimethyl-1,3-propanediamine in 50 mL of EtOH was added a solution of 2.96 g (25.6 mmol) of 2-chloroethylamine in 50 mL of EtOH

dropwise over 40 min. The solution was stirred at room temperature for 20 h. The solvent was evaporated and the residue was extracted with 2 x 50 mL of CH₂Cl₂, dried over Na₂SO₄, and evaporated to dryness. The resultant oil (1.52 g, 63%) was distilled (bp 145-148, 1 mm). Mass spectral analysis showed a m/e = 189 (M⁺ + 1). ¹H NMR (CDCl₃): δ 1.20 (4H, s), 1.60 (2H, quin), 2.29 (6H, s) 2.57 (4H, t), 2.73 (8H, t).

Example 6

[2-(bicyclo[3.3.1]non-3-ylamino)ethyl](3-{2-(bicyclo[3.3.1]non-3-ylamino)ethyl}amino)propylamine [Figure 6].

A mixture of 0.06 mol of 1-bromoadamantane and 0.30 mol of acetylated 2,3,2-tetramine were heated in a stainless steel bomb at 215 °C for 6 h. The product was poured into a mixture of 250 mL of 2 N HCl and 200 mL of ether. The aqueous layer was separated and made alkaline with 200 mL of 50% aqueous NaOH. The mixture was extracted with ether and the extract dried over K₂CO₃ and evaporated to give an oil (1.32 g, 33%). Mass spectral analysis showed a m/e = 406. ¹H NMR (CDCl₃): δ 1.24-1.30 (4H, s), 1.50-2.12 (32H, m), 2.62 (4H, t), 2.75 (8H, t).

Example 7

(2-aminoethyl){3-[(2-aminoethyl)amino]-1-methylbutyl}amine [Figure 7].

A mixture of 2.34 g (10 mmol) of 2,4-dibromopentane and 50 mL of absolute EtOH was added slowly to 1.2 g (20 mmol) of 1,2-diaminoethane hydrate. The mixture immediately became warm. It was then heated to 50 °C for 1 hour, 10 g of KCl added and the heating

continued for 30 minutes. The mixture was filtered from the KBr and distilled at reduced pressure. The compound was purified by converting it to its hydrochloride salt by addition of HCl. The melting point of the salt was $> 300\text{ }^{\circ}\text{C}$. It was converted back to its free amine by treatment with NH_4OH . Mass spectral analysis of the oil ((1.28 g, 68%) showed a $m/e = 188$. $^1\text{H NMR}$ (CDCl_3): δ 1.12 (6H, d), 1.30-1.37 (6H, s), 1.60 (2H, t), 2.60 (2H, m), 2.74 (8H, t).

Example 8

(2-pyridylmethyl){3-[(2-pyridylmethyl)amino]propyl}amine [Figure 8].

To a solution of 1.0 g (0.0135 mol) of 1,3-diaminopropane in 50 mL of EtOH was added a solution of 4.43 g (27.0 mmol) of 2-chloromethylpyridine in 25 mL of water. 10% NaOH was added until the pH reached 9. The solution was stirred at room temperature and NaOH was added to keep the pH at 8-9 over 3 days. The solvent was evaporated and the residue was extracted with 3 x 30 mL of CH_2Cl_2 , dried over Na_2SO_4 , and evaporated to dryness. The resultant oil (2.63 g, 76%) was analyzed. Mass spectral analysis showed a $m/e = 257$ ($\text{M}^+ + 1$). $^1\text{H NMR}$ (CDCl_3): δ 1.60 (2H, quin), 2.62 (4H, t), 4.06 (4H, s), 7.15-7.80 (6H, m), 8.44-8.63 (2H, d).

Example 9

methyl(3-[methyl(2-pyridylmethyl)amino]propyl)(2-pyridylmethyl)amine [Figure 9].

To a solution of 1.0 g (0.0128 mol) of N,N-dimethyl-1,3-propanediamine in 50 mL of EtOH was added a solution of 4.19 g (25.6 mmol) of 2-chloromethylpyridine in 25 mL of water. 10% NaOH was added until the pH reached 9. The solution was stirred at room

temperature and NaOH was added to keep the pH at 8-9 over 3 days. The solvent was evaporated and the residue was extracted with 3 x 30 mL of CH₂Cl₂, dried over Na₂SO₄, and evaporated to dryness. The resultant oil (2.69 g, 74%) was analyzed. Mass spectral analysis showed a m/e = 285 (M⁺ + 1). ¹H NMR (CDCl₃): δ 1.55 (2H, quin), 2.30 (6H, s), 2.58 (4H, t), 3.75 (4H, s), 7.07-7.85 (6H, m), 8.50-8.62 (2H, d).

Example 10

[2-(dimethylamino)ethyl](3-{[2-(dimethylamino)ethyl]methylamino}propyl)methylamine

[Figure 10].

A solution of 1.0 g (6.23 mmol) of 2,3,2-tetramine, 10 mL of formic acid, 10 mL of 37% formaldehyde and 1 mL of water was refluxed for 20 h. The solvent was evaporated, the solution was made basic with 3 M NaOH, and was extracted with 3 x 30 mL of CH₂Cl₂, dried over Na₂SO₄, and evaporated to dryness. The resultant oil (0.88 g, 58%) was analyzed. Mass spectral analysis showed a m/e = 244 (M⁺ + 1). ¹H NMR (CDCl₃): δ 1.62 (2H, quin), 2.24-2.30 (18H, s), 2.60 (4H, t), 2.71-2.75 (8H, t).

Example 11

2-[3-(2-aminoethylthio)propylthio]ethylamine [Figure 11].

To a solution of 1.0 g (0.0128 mol) of 1,3-dimercaptopropane in 50 mL of EtOH was added a solution of 1.48 g of NaOH in 10 mL of water. To the solution was added 214 g (18.48 mmol) of 2-chloroethylamine in 25 mL of EtOH. The solution was refluxed for 8 h. The solvent was evaporated and the residue was extracted with 3 x 25 mL of CH₂Cl₂, dried

over Na₂SO₄, and evaporated to dryness. The resultant oil was distilled at 165-173 (1 mm) to give 1.81 g, 73%. Mass spectral analysis showed a m/e = 194. ¹H NMR (CDCl₃): δ 1.48 (4H, s), 2.34-2.86 (14H, m).

Example 12

1,4,8,11-tetraaza-1,4,8,11-tetramethylcyclotetradecane [Figure 12].

A solution consisting of 1.0 g (0.005 mol) of cyclam, 5.3 mL of formic acid, 4.5 mL of 37% formaldehyde and 1 mL of water was refluxed for 18 h. The reaction mixture was transferred with 6 mL of water to a beaker and cooled to 5 °C in an ice bath. While stirring, a concentrated solution of NaOH was slowly added to pH >12. The temperature was kept below 25 °C during the addition and then extracted with 3 x 30 mL of CH₂Cl₂, dried over Na₂SO₄, and evaporated to dryness. The resultant oil (0.98 g, 71%) was analyzed. Mass spectral analysis showed a m/e = 256. ¹H NMR (CDCl₃): δ 1.68 (4H, quin), 2.22 (12H, s), 2.64 (8H, t), 2.75 (8H, t).

Example 13

1,4,8,11-tetraaza-1,4,8,11-tetra(2-piperidylethyl)cyclotetradecane [Figure 13].

To a solution of 0.5 g (2.5 mmol) of cyclam in 25 mL of CH₂Cl₂ was added a solution of 0.8 g of NaOH in 25 mL of water. A solution of 1.83 g (9.98 mmol) of 1-(2-chloroethyl)piperidine in 25 mL of CH₂Cl₂ was added dropwise at room temperature. The stirring was continued for 24 h. The solvent was evaporated and the residue was extracted with 3 x 50 mL of CH₂Cl₂, dried over Na₂SO₄, and evaporated to dryness. The resultant oil (0.725

g, 45%) was analyzed. Mass spectral analysis showed a $m/e = 646 (M^+ + 1)$. ^1H NMR (CDCl_3): δ 1.28 (8H, q), 1.46-1.72 (24H, m), 1.72 (4H, m), 2.42-2.80 (24H, m), 2.64 (8H, t), 2.75 (8H, t).

Example 14

1,4,8,11-tetraaza-1,4,8,11-tetrabicyclo[3.3.1]non-3-ylcyclotetradecane [Figure 14].

To 0.5 g (2.5 mmol) of cyclam in 50 mL of EtOH was added 2.15 g (10.0 mmol) of 1-bromoadamantane in 50 mL of EtOH dropwise over 30 min. The solution was heated to reflux and heated for 20 h. The solution was evaporated under reduced pressure, extracted with 3 x 35 mL of CH_2Cl_2 , dried over Na_2SO_4 , and evaporated to dryness. The resultant oil (0.53 g, 31%) was analyzed. Mass spectral analysis showed a $m/e = 690 (M^+ + 1)$. ^1H NMR (CDCl_3): δ 1.24-1.58 (56H, m), 1.66 (4H, quin), 2.62 (8H, t), 2.70 (8H, t).

Example 15

1,4,8,11-tetraaza-1,4,8,11-tetraethylcyclotetradecane [Figure 15].

To a stirred solution of 1.0 g (5.0 mmol) of cyclam in 50 mL of DMF was added 4.0 g (0.1 mol) of NaH in small portions. The solution was heated under nitrogen at 60°C for 3 h. 3.12 g (20 mmol) of iodoethane was added in one portion. The solution was heated at 60°C for 18 h. The reaction was quenched with 95% EtOH, extracted with 3 x 35 mL of CH_2Cl_2 , dried over Na_2SO_4 , and evaporated to dryness. The resultant oil was purified by flash chromatography using ethyl acetate/MeOH. Mass spectral analysis of the oil (0.72 g, 46%)

showed a $m/e = 312$. ^1H NMR (CDCl_3): δ 1.38 (12H, t), 2.16 (8H, q), 3.38 (4H, quin), 3.54 (8H, t), 3.80 (8H, t).

Example 16

$\text{N,N}'$ -(2'-dimethylphosphinoethyl)-propylenediamine [Figure 16].

Propylenediamine (4.0 g) was dissolved in 200 mL of ethanol. To the solution was added 9.4 g of dimethylvinylphosphine sulfide and the mixture was heated at reflux for 72 h. The solvent was evaporated under reduced pressure and the residue dissolved in 400 mL of chloroform and washed with 50 mL of 2 M NaOH and dried over MgSO_4 . The solvent was removed under reduced pressure to give an oil that was crystallized from ethyl acetate to give 6.8 g (51%) of the pure product. To a suspension of LiAlH_4 (1.2 g) in 125 mL of dry dioxane was added $\text{N,N}'$ -(2'-dimethylphosphinoethioethyl)-propylenediamine (prepared as above). The mixture was refluxed for 36 h. The mixture was cooled, dioxane/water added, 3 mL of 2 M NaOH added and then the solution was filtered to give the pure phosphine. ^1H NMR (CDCl_3): δ 1.64(2H, quin), 2.10(12H,s), 2.57(4H, t), 2.55-2.80(8H,m).

Example 17

Vanadyl 2,3,2-Tetramine [Figure 17].

To 1.0 g (0.0624 mol) of 2,3,2 tetramine in 20 ml of EtOH was added 0.073 g (0.0624 mol) of vanadylacetylacetonate in 20 ml of EtOH. The solution was refluxed for 30 min and cooled to room temperature. Overnight a red-brown solid precipitated. The complex is formulated as being $[\text{VO}(2,3,2\text{-tetramine})\text{acac}]$.

Example 18

Chromium 2,3,2-Tetramine [Figure 18].

To 1.0 g (0.0624 mol) of 2,3,2-tetramine in 20 ml of EtOH was added 0.245 g (0.0624 mol) of chromium (III) nitrate in 20 ml. Of EtOH. The solution was refluxed for 30 min and cooled to room temperature. Overnight a solid precipitated. The complex is formulated as being $[\text{Cr}(2,3,2\text{-tetramine})(\text{NO}_3)_2]\text{NO}_3$.

Example 19

Comparison of Stabilities of Metal Ion Complexes

Referring to the heats of formation, the first modification to consider is how the heats of formation are affected by changing the metal ion. The data is quite clear here with the relative stabilities following the pattern: $\text{Co} > \text{Fe} > \text{Mn} > \text{Cu} > \text{Zn} > \text{Cd}$. Occasionally the Cu complexes are more stable than the Mn but otherwise the trend holds consistently from one set of complexes to another. The trend in changes in stability due to changes in the metal may be exploited by recognizing the affinity that the organic compounds have for various metal ions in the body.

In Table III it is apparent that Fe 2,3,2 -piperidine and Fe 2,3,2- adamantane have a low heat of formation which would be attractive to address the excess iron pools in neurodegenerative disease and the excess iron released into brain tissue following lysis of dead neurons post stroke, the adamantane having additional effect on the NMDA receptor.

Similarly from Tables II and IV it is apparent that Fe cyclam methylated and Fe cyclam adamanatane are very stable and Zn cyclam methylated and Zn cyclam adamantane not unduly

stable. This behavior could be useful in treating ischemic damage post myocardial infarction where iron exerts toxic redox effects and tissue zinc stores are rapidly depleted.

From Tables II and V it is apparent that there are open ring molecules binding copper and manganese, Cu 2,3,2-isopropyl on N1/N4 and Mn 3,3,3 respectively are as stable as closed ring molecules. Thus open ring molecules are comparable with closed ring molecules in their capacity to address free metal excess in neurodegenerative diseases and stroke.

Example 20

Ring Size

For the 2,3,2-tetramine compounds, formation of 6-membered rings when binding to metal ions increases the stability of the metal complexes. This can be seen when comparing the 3,3,3-tetramine metal complexes to the corresponding 2,3,2-tetramine and the 2,2,2-tetramine compounds. In all cases, the 3,3,3-tetramine complexes are more stable than their 2,3,2-tetramine counterparts. Also, it is generally true that the 2,3,2-tetramine complexes are more stable than the 2,2,2-tetramine complexes. This suggests that the 3,3,3-tetramine compounds may be of considerable interest as companions to the 2,3,2-tetramine compounds. Schugar H. and coworkers (Inorg. Chem., 19, 940, 1980) have shown through stability constants that changing the size of the chelate ring has an effect on the resultant stability of the metal complex.

Modification of the cyclam rings so that the rings are smaller or larger also impacts the heats of formation. The cyclen complexes are less stable than the cyclam rings, a result that has been documented elsewhere. Increasing the size of the ring as was done for the cyclam 3,3,3-tetramine complexes also leads to enhanced stability compared to cyclam.

These size related changes in stability influence the design of compounds for treatment of neurodegenerative disorders, stroke, glaucoma, Atherosclerosis, cardiomyopathy, ischemia, optic neuropathy, peripheral neuropathy, Presbycusis and cancer.

Example 21

Addition of Side Groups on Open Ring Molecules

Along with changing the size of the rings, various alkyl groups were put on the nitrogens or carbons to see how these modifications affected the stability of the complexes. A number of generalizations can be extracted from the data. First, putting small alkyl groups on the nitrogens generally enhances their stability. This can be seen when comparing the 2,3,2-tetramine compounds to the ones where either N1/N4 or N2/N3 are substituted with methyl groups. This result also holds for isopropyl groups substituted on N1/N4 and generally for isopropyls on N2/N3. There is a limit to adding large groups on the nitrogens as seen by the compounds with benzyl substituents. These complexes are very much less stable than the unsubstituted 2,3,2-tetramine complexes.

The placement of alkyl groups on the carbons was only studied in a few cases, but for all of them the addition of methyls led to enhanced stability at a level comparable to that found when the methyls were placed on the nitrogens.

Addition of Side Groups on Closed Ring Molecules

The trends for the heats of formation of the cyclam complexes are not as consistent as those found for the 2,3,2-tetramine complexes. For example, putting methyl groups on the nitrogens increases the stability in some cases but decreases it in others. The same is true for the complexes where isopropyl is added to the nitrogens. Once again though, benzyl groups greatly decrease the stability of the complexes showing that there is an upper limit as to how bulky the substituents can be before the stability of the complexes is greatly diminished. Surprisingly, the addition of adamantane to the cyclams leads to enhanced stability in all cases. Adamantane is a very large group but it is able to find ways to exist so that the structure is actually quite stable. This stability of the cyclam adamantane compounds may be useful in situations such as stroke and glaucoma where NMDA receptor antagonism is required.

From a biopassaging standpoint the stability of the 2,3,2-isopropyl complexes and molecules with carbon side chains attached to the ring nitrogens or carbons is valuable toward developing compounds which are more lipophilic and thus have better passage across the gastrointestinal tract, blood brain barrier and blood retinal barrier, this being important in the treatment of Parkinson's, Alzheimer's, Lou Gehrig's, Binswanger's, Lewy Body diseases, Olivopontine Cerebellar Degeneration, Stroke, Glaucoma and Optic Neuropathy described herein.

Example 22

Modifications of Terminal Nitrogens

Another important result is the one shown by changing N1/N4 into piperidine or piperazine nitrogens. It should be noted that these compounds are somewhat different than the ones described above in that the piperidine groups are not added to N1/N4 but rather N1/N4 are replaced by the piperidine or piperazine. With the exception of the copper complexes, these complexes are more stable than the base 2,3,2-tetramine complexes. No generalizations can be made regarding the adamantane compounds but it is noteworthy that they are not excessively unstable compared to the 2,3,2-tetramine compounds (indeed, the Fe complex is more stable while the Co one is equal in stability) even though they are quite large and bulky. This suggests that even large, bulky alkyl groups placed on the nitrogens may not adversely affect their properties and they should be pursued.

The piperidine, piperazine and adamantane derivative molecules are attractive because the terminal groups can substantially alter basicity, lipophilicity and passage through membranes, in addition to altering receptor binding properties. These derivatives may also be attractive where a selective bias towards iron removal versus stored copper removal is sought. This could be applicable to therapeutics for ischemia post myocardial infarction, atherosclerosis and neurodegenerative diseases.

Further the stability of terminally substituted derivatives provides opportunity for substitution with glutathione, uric acid, ascorbic acid, taurine, estrogen, dehydroepiandrosterone, probucol, vitamin E, hydroxytoluene, carvidilol, α -lipoic acid, α -tocopherol, ubiquinone, phyloquinone, β -carotene, meanadione, glutamate, succinate, acetyl-

L-carnitine, co-enzyme Q, lazeroids, and polyphenolic flavonoids or homocysteine, menaquinone, idebenone, dantrolene.

These specific derivatives of polyamines may be used as compounds in the treatment of, though not limited to, the following diseases:

glutathione polyamine in peripheral neuropathy and ischemia

uric acid polyamine in stroke

ascorbic acid polyamine in diabetic neuropathy and ischemia

taurine polyamine in diabetic neuropathy

estrogen polyamine in stroke

dehydroepiandrosterone polyamine in stroke

probulcol polyamine in peripheral neuropathy

vitamin E polyamine in peripheral neuropathy, Alzheimer's disease, stroke and ischemia,

hydroxytoluene polyamine in peripheral neuropathy

carvidilol polyamine in peripheral neuropathy

α -lipoic acid polyamine in presbycusis, peripheral neuropathy and diabetic neuropathy and Alzheimer's disease

α -tocopherol polyamine in atherosclerosis and ischemia

menaquinone polyamine in diabetes

ubiquinone polyamine in ischemia

phyllloquinone (Vitamin K₁) polyamine in atherosclerosis and cardiomyopathy

β -carotene polyamine in ischemia

glutamate polyamine in diabetes

succinate polyamine in diabetes

acetyl-L-carnitine polyamine in Alzheimer's disease and presbycusis
co-enzyme Q polyamine in diabetes, cardiomyopathy and congestive heart failure
lazeroid (21 aminoquinone) polyamine in stroke
polyphenolic flavonoid (quercetin, catechin, epicatechin) polyamine as antioxidants
homocysteine polyamine in cancer
meanadione (Vitamin K₃) polyamine in cardiomyopathy
idebenone polyamine in cardiomyopathy, MELAS and stroke
dantrolene polyamine in stroke
Memantine polyamine, rimantidine polyamine in glaucoma.

Example 23

Internal Substitutions in the Open Ring Molecules

It is also possible to replace the nitrogens with other donors such as sulfur. As shown, these complexes excepting the iron ones are considerably more stable than the nitrogen ones. Sulphur containing polyamines terminally derivatized with homocysteine could be used as anti-cancer agents.

Internal Substitutions in the Closed Ring Molecules

Replacing the nitrogens with sulfur enhances the stability of some complexes (Cu, Zn, Co) but not in others (Fe, Mn). This result shows that it is possible to build into the organic compounds selectivity for some metal ions over others. Again a sulphur containing closed ring polyamine derivatized with homocysteine could be used as an anti-cancer agent.

Example 24

Pharmacokinetic advantages versus stability of derivatives

Terminal modifications and side chain additions alter pKa, lipophilicity and also the metabolism of these compounds, thus changing half life in vivo. 2,2,2-tetramine is rapidly metabolized to acetyl 2,2,2-tetramine and rapidly excreted with a half life in vivo of only a few hours (Kodama H. et al 1997). This metabolism will obviously be altered considerably in terminally derivatized compounds and to some extent in molecules with side chains attached and in internally derivatized molecules. In the treatment of the diseases mentioned above a longer half life and less frequent dosing such as once daily dosing will be highly advantageous for therapeutic effect and patient compliance.

Additional Comments

The results in Tables I to VIII shed light on the stability of these molecules and helps direct which ones are appropriate for particular disease situations based upon metal ion selectivity and pharmacological actions and how to enhance the bioavailability of orally or parenterally delivered drugs, and drugs crossing particular membranes such as the blood brain barrier and blood retinal barrier.

Example 25
Oil Water Partition Coefficients

Partition coefficients were determined by dissolving the compound in a 1:1 mixture of octanol/water and shaking the solution for 12 hours. HPLC was used to determine the partition coefficient. The reported values are the log of the octanol/water partition

Table IX. Oil Water Partition Coefficients

Compound	Log Partition Coefficient Octanol : Water
2,2,2-tetramine	1.6
2,3,2-tetramine	2.1
2,3,2-pyridine	2.7
2,3,2-CH ₃ on N1/N4	0.4
cyclam-piperidine	0.7

Octanol : water partition log partition coefficients of 2 are optimal for passage through lipid membranes and tissue barriers. Molecules within a range from 0.5 to 4.0 are potential candidates for in vivo use. Thus 2,2,2-tetramine, 2,3,2-tetramine and 2,3,2-pyridine have optimal lipid water partitioning to facilitate their passage through the gastrointestinal barrier and the blood brain barrier.

Example 26

PKa's

PKa's were determined by standard potentiometric titration methods in aqueous solution with an ionic strength of 0.10 at 25 °C. Values are reported as log K values of the equilibrium constant.

Table X. pKa's

	pKa(1)	pKa(2)	pKa(3)	pKa(4)
2,2,2-tetramine	9.7	9.1	6.6	3.3
2,3,2-tetramine	10.3	9.5	7.3	6.0
2,3,2-pyridine	8.3	7.4		
2,3,2-piperidine	9.9	9.3	6.4	3.6
2,3,2-tetramethyl	10.2	9.4	6.1	2.9
tetramethylcyclam	9.7	9.3	3.1	2.6

2,3,2 -pyridine is less basic and thus more soluble at neutral pH than some of the other amines. Selection of compounds with appropriate pKa's for use in various diseases where low pKa's would be useful. Selection of compounds with appropriate pKa's for use in various diseases where higher pKas would be useful such as in diabetes and post myocardial infarction.

Example 27

Diseases and individual mechanisms of action

Following are examples of therapeutic actions of polyamines in various diseases:

Neurodegenerative diseases- Parkinson's disease, Alzheimer's disease, Lou Gehrig's disease, Binswanger's disease, Olivopontine Cerebellar Degeneration, Lewy Body disease.

Polyamines treat these diseases by;

a) Competitive inhibition of uptake of xenobiotics at the polyamine transport site, such organic molecules being a cause of depigmentation and DNA damage; b) Steric shielding of DNA from organic molecules by compacting DNA; c) Limitation of mitochondrial DNA damage by removal of free copper, iron, nickel, mercury and lead ions by the presence of a polyamine; d) Induction of metallothionein gene transcription; e) Induction of nerve growth factor, brain derived neuronotrophic factor and neuronotrophin-3 gene transcription; f) Regulation of affinity of NMDA receptors and blockade of the MK801 ion channel; g) Inhibition of protein kinase C; h) Mitochondrial reuptake of calcium; i) Binding and conservation of reduced glutathione; j) Induction of ornithine decarboxylase by glutathione; k) Maintenance of the homeostasis of the redox environment in brain; l) Non toxic chelation of divalent metals in brain; m) Regulation of activity of preaspartate proteases; n) Inhibition of acetylcholinesterase and butyrylcholinesterase; o) Blockade of muscarinic M_2 receptors; p) Maintenance of ratio of membrane phosphatidylcholine: phosphatidylserine ratio; q) Inhibition of superoxide dismutase, amine oxidase, monoamine oxidase B by binding of free copper; r) Regulation of brain polyamine levels in dementias with maintenance of endogenous polyamine levels; s) Blockade of neuronal n and p type calcium channels. To treat neurodegenerative diseases require prevention of mitochondrial DNA damage, maintenance of the oxidative phosphorylation activity of cells, induction of cellular repair mechanisms, regulation of receptor and enzymatic activities.

Diabetes Mellitus

Age, growth and metabolic requirements, weight and body mass, predisposition to atherosclerotic and vascular complications influence the treatment selection for diabetes mellitus patients.

Several drugs may be developed to treat Type I and Type II diabetes mellitus and its vascular and neuronal complications, treatment choices being related to age, weight, body mass and clinical stage of disease; compositions which provide mitochondrial protection; compositions which additionally increase insulin output, compositions which enhance glucose tolerance, compositions which reduce insulin requirements and compositions which prevent diabetic nephropathy:

Mitochondrial Protection

a) Competitive inhibition of uptake of xenobiotics at the polyamine transport site, such organic molecules being a cause of mitochondrial DNA damage; b) Steric shielding of DNA from organic molecules by compacting DNA; c) Limitation of mitochondrial DNA damage by removal of free copper, iron, nickel, mercury and lead ions by the presence of a polyamine; d) Induction of metallothionein gene transcription; e) Inhibition of protein kinase C; f) Mitochondrial reuptake of calcium; g) Binding and conservation of reduced glutathione; h) Induction of ornithine decarboxylase by glutathione; i) Maintenance of the homeostasis of the redox environment j) Inhibition of superoxide dismutase, amine oxidase by binding of free copper. Succinate and glutamate derivatized polyamines can stimulate insulin release. Prevention of mitochondrial DNA damage, maintaining oxidative phosphorylation, maintaining mitochondrial membrane integrity from free radical induced damage and stimulating insulin secretion via exocytosis or reducing insulin secretion in states of hyperinsulinism are important objectives in the treatment of diabetes.

Enhancing Insulin Release

Succinate polyamines increase the supply of succinic acid and acetyl CoA to the Krebs cycle they stimulate insulin synthesis and release they increase insulin output at high concentrations of glucose. Glutamate polyamines stimulate release of insulin by promoting exocytosis.

However in forms of diabetes associated with hyperinsulinism further insulin secretion is not desired because it may further damage β islet cells thus causing islet amyloid deposition and it contributes to macrovascular damage. Agents which increase glucose tolerance whilst not increasing insulin output can be helpful in managing the disease. Chromium and vanadium polyamine complexes are useful in that regard.

Obesity and Hyperinsulinism and Lipid Balance

A chromium polyamine complex can deliver trivalent chromium to its target sites where it promotes glucose tolerance in instances where body mass index is greater than average. A trivalent chromium polyamine complex can enhance glucose tolerance and decrease blood cholesterol and triglycerides, and increase high density lipoprotein in diabetics with above average body mass index and in obese patients having incipient diabetes. A chromium polyamine combines mitochondrial protection with enhanced glucose tolerance and metabolic regulation of lipid and carbohydrate metabolism.

Reducing Insulin Requirement, Carbohydrate Absorption and Maintaining Lipid Balance

Tetravalent vanadium polyamine complexes may be used in Type I and Type II diabetes to achieve metabolic control and diminish insulin requirement. A vanadyl polyamine complex delivers vanadium in its cationic vanadyl V(IV) form to the tissues and a smaller dose of vanadium is required than when administered in other salt forms. Vanadium decrease blood glucose and D-3-hydroxybutyrate levels in diabetes, it also restores fluid intake and body weight of diabetic animals. These metabolic effects occur because vanadium a) decreases P-enolpyruvate carboxykinase (PEPCK) transcription, thus decreasing gluconeogenesis; b) it decreases tyrosine aminotransferase gene expression; c) it increases expression of glucokinase gene; d) it induces pyruvate kinase; e) it decreases mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (HMGCoAS) gene expression; f) it decreases the expression of the liver and

pancreas glucose-transporter GLUT-2 gene in diabetic animals to the level seen in controls; g) it increases the amount of the insulin-sensitive glucose transporter, GLUT4 by stimulating its transcription; h) the insulin like metabolic effects of vanadium are mediated by inhibition of protein tyrosine phosphatases (PTP). Peroxovanadium compounds irreversibly oxidize the thiol group of the essential cysteine at the PTP catalytic site. Vanadium is a structural analog of phosphate. Vanadium does not exhibit the growth effects and mitogenic effects of insulin and thus might avoid the macrovascular diseases consequences of hyperinsulinemia and be clinically useful in disease where insulin resistance is caused by defects in the insulin signaling pathway. Vanadium mimics the effects of insulin in restoring G proteins and adenylyl cyclase activity increasing cyclic AMP levels; i) vanadyl ion suppresses nitric oxide production by macrophages; j) it has a positive cardiac inotropic effect; k) vanadium restores albumin mRNA levels in diabetic animals by increasing hepatic nuclear factor 1 (HNF 1); l) it restores triiodothyronine T₃ levels. Vanadyl polyamine has the advantages of mitochondrial protection combined with the ability to regulate the insulin signaling pathways, with effects on glucose, carbohydrate and fat metabolism. It can lower insulin requirements, thus overcoming the vascular consequences of hyperinsulinism, permit viable β cells to continue functioning and will exert these functions irrespective of body mass index.

Diabetic Nephropathy

Polyamines which more potently decrease protein kinase C activity than others may be used in the treatment of diabetic nephropathy. Protein kinase C causes apoptosis in diabetic nephropathy and polyamines reduce protein kinase C activation. Protein kinase C is overactivated due to excess diacylglycerol (DAG) formation from glucose.

Stroke

Polyamines treat the consequences of stroke in the following manner;

a) Induction of metallothionein gene transcription; b) Induction of nerve growth factor, brain derived neuronotrophic factor and neuronotrophin-3 gene transcription; c) Regulation of affinity of NMDA receptors and blockade of the MK801 ion channel; d) Inhibition of protein kinase C; e) Mitochondrial reuptake of calcium; f) Binding and conservation of reduced glutathione; g) Induction of ornithine decarboxylase by glutathione; h) Maintenance of the homeostasis of the redox environment in brain; i) Non toxic chelation of divalent metals in brain; j) Inhibition of superoxide dismutase and amine oxidase k) Regulation of brain polyamine levels in dementias with maintenance of endogenous polyamine levels; l) Blockade of neuronal n and p type calcium channels.

Prevention of oxidative damage during the reperfusion post ischemia and removal of redox metals released from dead cells, trapped in the tissue are important objectives.

Atherosclerosis, Myocardial Ischemia, Cardiomyopathy, Ischemia, Optic Neuropathy, Peripheral Neuropathy

Polyamines treat atherosclerosis onset and progression by the following mechanisms;

a) Steric shielding of DNA from organic molecules by compacting DNA; b) Limitation of mitochondrial DNA damage by removal of free copper, iron and cadmium ions by the presence of a polyamine; c) Induction of metallothionein gene transcription; d) Inhibition of protein kinase C; e) Mitochondrial reuptake of calcium; f) Binding and conservation of reduced glutathione; g) Induction of ornithine decarboxylase by glutathione; h) Maintenance of the homeostasis of the redox environment; i) Inhibition of superoxide dismutase and amine oxidase by binding of free copper. Prevention of mitochondrial DNA damage, maintaining

oxidative phosphorylation, maintaining normal LDL : HDL lipid ratios and preserving mitochondrial membrane integrity from free radical damage are major objectives in these diseases. In atherosclerosis prevention of oxidation of low density lipoprotein is also important.

The chromium and vanadium polyamines mentioned above in relation to diabetic treatment are useful with regards to improving lipoprotein ratios and preventing atherosclerotic plaque formation.

Glaucoma

Polyamines treat glaucoma by;

- a) Limitation of mitochondrial DNA damage by removal of free metals by the presence of a polyamine; b) Induction of metallothionein gene transcription; c) Regulation of affinity of NMDA receptors and blockade of the MK801 ion channel; d) Mitochondrial reuptake of calcium; e) Binding and conservation of reduced glutathione; f) Induction of ornithine decarboxylase by glutathione; g) Maintenance of the homeostasis of the redox environment; h) Non toxic chelation of divalent metals; i) Inhibition of superoxide dismutase and amine oxidase by binding of free copper; j) Regulation of polyamine levels in M ganglion cells with maintenance of endogenous polyamine levels. The M ganglion cells are pigment and metal rich and very prone to glutamate toxicity.

Presbycusis

Polyamines treat presbycusis by;

10017235-121801

5 a) Steric shielding of DNA from organic molecules by compacting DNA; b) Limitation of mitochondrial DNA damage by removal of free copper and iron ions by the presence of a polyamine; c) Induction of metallothionein gene transcription; d) Inhibition of protein kinase C; e) Mitochondrial reuptake of calcium; f) Binding and conservation of reduced glutathione; g) Induction of ornithine decarboxylase by glutathione; h) Maintenance of the homeostasis of the redox environment; i) Inhibition of superoxide dismutase and amine oxidase by binding of free copper. a, b) and c) prevent mitochondrial DNA damage which increases in the cochlea during aging and causes deafness.

10 **Cancer.**

Polyamines form extremely stable complexes with cobalt as indicated by their heats of formation. A cobalt dihomocysteine polyamine complex can behave like thioretinaco. As a non toxic, intracellular electrophile it will promote ATP formation and protect against free oxygen species produced by toxins, radiation and cancer cells. Further it would diminish homocysteic acid formation, which promotes growth factor activity, and thus prevent the 15 invasiveness and neovascularization caused by cancer cells.

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References Cited

Abraham A.S. Brooks B.A., Eylath U. The effects of chromium supplementation on serum glucose and lipids in patients with and without non-insulin-dependent diabetes. *Metabolism: Clin. Exper.* (1992) Jul, 41(7):768-71.

5

Abraham C.R., Driscoll J., Potter H., Van Nostrand W.E., Tempst P. A calcium-activated protease from Alzheimer's disease brain cleaves at the N-terminus of the amyloid B-protein. *Biochem. Biophys. Res. Commun.* (1991), 174, 790 - 96.

10

Abraham C.R., Razzaboni B.L., Sisodia S.S., Koo E.H., Price D.L., Van Nostrand W.E., Papastoitsis G. Studies on the proteolytic degradation of the B-protein precursor by proteases purified from Alzheimer's brain. *Ann. New York Acad. Sci.* (1991), 640, 161 - 65.

15

Abraham C.R., Razzaboni B.I., Papastoitsis G., Picard E., Kanemaru K., Meckelein B., Mucke L. Purification and cloning of brain proteases capable of degrading the B-amyloid precursor protein. *Ann. New York Acad. Sci.* (1992) 674, 174 - 179.

20

Akkan A.G., Malaisse W.J. Iterative pulse administration of succinic acid monomethyl ester to streptozotocin diabetic rats. *Diabetes Res.* (1993), 23(2):55-63.

24

Aldrich, P., Hermann, E.C., Meier, W. E., Antiviral Agents. Structure-Activity Relationships of Compounds Related to 1-Adamantanamine, *Jour. Med. Chem.*, (1971), 14, 535-543.

Anand-Srivastava M.B., McNeill J.H. Yang X.P. Reversal of defective G-proteins and adenylyl cyclase/cAMP signal transduction in diabetic rats by vanadyl sulphate therapy. *Molec. Cell. Biochem.* (1995), Dec 6-20, 153(1-2):113-9.

Arbustini, E; Diegoli, M; Fasani, R; Grasso, M; Morbini, P; Banchieri, N; Bellini, O; Dal Bello, B; Pilotto, A; Magrini, G; Campana, C; Fortina, P; Gavazzi, A; Narula, J; Vigano, M. Mitochondrial DNA mutations and mitochondrial abnormalities in dilated cardiomyopathy. *Amer. Jour. Path.* (1998), Nov, 153(5), 1501-10.

Arbustini, E; Morbini, P; Pilotto, A; Gavazzi, A; Tavazzi, L. Genetics of idiopathic dilated cardiomyopathy. *Herz* (2000), May, 25(3), 156-60.

Baeza I., Ibanez M, Wong C., Chavez P., Gariglio P. Possible prebiotic significance of polyamines in the condensation, protection, encapsulation, and biological properties of DNA. *Orig. Life Evol. Spec.* (1992), 21, 225 - 42.

Barefield, K., Wagner, F., Metal Complexes of 1,4,8,11-Tetramethyl-1,4,8,11-tetraazacyclotetradecane, N-Tetramethylcyclam, *Inorg. Chem.*, (1973), 12, 2435-2436.

Barefield, E.K., Wagner, F., Hodges, K.D., Synthesis of Macrocyclic Tetramines by Metal Ion Assisted Cyclization Reactions. *Inorg. Chem.*, (1976), 15, 1370-1377.

Barrera-Hernandez G., Wanke I.E., Wong N.C. Phlorizin or vanadate treatment reverses impaired expression of albumin and hepatocyte nuclear factor 1 in diabetic rats. *Diabetes* (1996), Sep, 45(9):1217-22.

Barrett, G.M., et al., Dissolving Metal Reduction of Esters to Alkanes. *Jour. Chem. Soc., Perkin I*, (1981), 1501-1509.

Beneviste M., Mayer M.L. Multiple effects of spermine on n-methyl-D-aspartic acid receptor responses of rat cultured hippocampal neurons. *Jour. Physiol.* (1993), 464, 131 - 63.

Black R.A., Kronheim S., Merriam J., March C., Hopp T.A. Preaspartate protease from human leukocytes that cleaves pro-interleukin-1B. *Jour. Biol. Chem.* (1989), 264, 5323 - 26.

Blomgren K., Nilsson E., Karlsson J. Calpain and calpastatin levels in different organs of the rabbit. *Compar. Biochem. Physiol. B.* (1989), 93, 403 - 07.

Bonte, CA; Matthijs, GL; Cassiman, JJ; Leys, AM. Macular pattern dystrophy in patients with deafness and diabetes. *Retina* (1997), 17(3):216-21.

Bradley W.G., Krasin F. A new hypothesis of the etiology of amyotrophic lateral sclerosis. *Arch. Neurol.* (1982), 39, 677 - 80.

Borthwick, GM; Johnson, MA; Ince, PG; Shaw, PJ; Turnbull, DM. Mitochondrial enzyme activity in amyotrophic lateral sclerosis: implications for the role of mitochondria in neuronal cell death. *Ann. Neurol.* (1999), Nov, 46(5):787-90.

5 Brabenboer B., Kappelle A.C. Harmers FPT, van Buren T., Erkelens D.W., Gispen W.H. Potential use of glutathione for the prevention and treatment of diabetic neuropathy in the streptozotocin-induced diabetic rat. *Diabetologia* (1992), 35, 813 – 17.

10 Brantl V., Gramsch C., Lottspeich F., Henschen A., Jager R.A., Herz A. Novel opioid peptides derived from mitochondrial cytochrome b: cytocorphins. *Eur. Jour. Pharmacol.* (1985), III, 293 - 94.

Bu, X, Rotter, J.I. Wolfram syndrome: a mitochondrial-mediated disorder? *Lancet*, (1993) Sep 4, 342(8871):598-600.

15 Bush, AI; Pettingell, WH; Multhaup, G; d Paradis, M; Vonsattel, JP; Gusella, JF; Beyreuther, K; Masters, CL; Tanzi, RE. Rapid induction of Alzheimer A β -amyloid formation by zinc [see comments] *Science* (1994), Sep 2, 265(5177):1464-7.

20 Cam M.C., Rodrigues B., McNeill J.H. Distinct glucose lowering and beta cell protective effects of vanadium and food restriction in streptozotocin-diabetes. *Eur. Jour. Endocrin.* (1999), Nov, 141(5):546-54.

Cam M.C., Pederson R.A., Brownsey R.W., McNeill J.H., Long-term effectiveness of oral vanadyl sulphate in streptozotocin-diabetic rats. *Diabetologia* (1993), 36, 218 – 24.

Cam M.C. Brownsey, RW, McNeill, JH. Mechanisms of vanadium action: insulin-mimetic or insulin-enhancing agent? *Can. Jour. Physiol. Pharmacol.* (2000) Oct, 78(10):829-47.

Cameron N.E., Cotter M.A., Maxfield E.K. Anti-oxidant treatment prevents the development of peripheral nerve dysfunction in streptozotocin-diabetic rats. *Diabetologia* (1993), 36, 299 – 304.

Cameron N.E., Cotter M.A., Archoibald V., Dines K.C., Maxfield E.K., Anti-oxidant and Pro-oxidant effects on nerve conduction velocity, endoneurial blood flow and oxygen tension in non-diabetic and streptozotocin-diabetic rats. *Diabetologia* (1994), 37, 449 – 59.

Cavanaugh P.F., Pavelic Z. P., Porter C.W. enhancement of 1,3-Bis(2-chloroethyl)-1-nitrosurea-induced cytotoxicity and DNA damage by α -difluoromethylornithine in L1210 leukemia cells. *Cancer Res.* (1984), 44, 3856 – 61.

Chakrabarti A.K., Banik N., Powers J., Hogan E. The regional and subcellular distribution of calcium activated neutral proteinase (CANP) in the bovine central nervous system. *Neurochem. Res.* (1989), 14, 259 - 66.

Chen H., Carlson EC., Pellet L., Moritz, J.T., Epstein P.N. Overexpression of metallothionein in pancreatic beta-cells reduces streptozotocin-induced DNA damage and diabetes. *Diabetes* 2001 Sep, 50(9):2040-6.

5 Chen J., Jin K., Chen M., Pei w., Kawaguchi K., Greenberg D.A., Simon R.P. early detection of DNA strand breaks in the brain after focal transient ischemia: implications for the role of DNA damage in apoptosis and neuronal cell death. *Jour. Neurochem.* (1997), 69, 232 – 45.

10017235.121801
10 Chen, SJ; Bradley, ME; Lee, TC. Chemical hypoxia triggers apoptosis of cultured neonatal rat cardiac myocytes: modulation by calcium-regulated proteases and protein kinases. *Molec. Cellul. Biochem.* (1998), Jan, 178(1-2), 141-9.

Chu P., CC; Huang, CC; Fang, W; Chu, NS; Pang, CY; Wei, YH. Peripheral neuropathy in mitochondrial encephalomyopathies. *Eur. Neurol.* (1997), 37(2):110-15.

15 Chu P., Saito H., Abe K. Polyamines promote regeneration of injured axons of cultured rat hippocampal neurons. *Brain Res.* (1995), 673, 233- 41.

20 Clark, A; de Koning, EJ; Hattersley, AT; Hansen, BC; Yajnik, CS; Poulton, J. Pancreatic pathology in non-insulin dependent diabetes (NIDDM). *Diabetes Res. Clin. Prac.* (1995), Aug, 28 Suppl., S39-47.

24 Clayton D.A., Doda J.N., Friedberg E.C. The absence of a pyrimidine dimer repair mechanism in mammalian mitochondria. *Proc. Natl. Acad. Sci.* (1974), 71, 2777 - 81.

Clopath P., Smith V.C., McCully K.S. Growth promotion by homocysteic acid. Science (1976), 373 – 74.

Cohen N., Halberstam M., Shlimovich P., Chang C.J., Shamoan H., Rossetti L. Oral vanadyl sulfate improves hepatic and peripheral insulin sensitivity in patients with non-insulin-dependent diabetes mellitus. Jour. Clin. Invest. (1995), Jun, 95(6):2501-9.

Comi, GP; Bordoni, A; Salani, S; Franceschina, L; Sciacco, M; Prella, A; Fortunato, F; Zeviani, M; Napoli, L; Bresolin, N; Moggio, M; Ausenda, CD; Taanman, JW; Scarlato, G. Cytochrome c oxidase subunit I microdeletion in a patient with motor neuron disease. Ann.Neurol. (1998), Jan, 43(1):110-6.

Corral-Debrinski, M; Shoffner, JM; Lott, MT; Wallace, DC. Association of mitochondrial DNA damage with aging and coronary atherosclerotic heart disease. Mutat. Res. (1992), Sep, 275(3-6), 169-80.

Corral-Debrinski M. Mitochondrial DNA deletions in human brain: regional variability and increase with advanced age. Nat. Genet. (1992), Dec;2(4):324-9.

Corral-Debrinski, M; Horton, T; Lott, MT; Shoffner, JM; McKee, AC; Beal, MF; Graham, BH; Wallace, DC. Marked changes in mitochondrial DNA deletion levels in Alzheimer brains. Genomics (1994), Sep 15, 23(2):471-6.

Cotter M.A., Cameron N.E. Neuroprotective effects of carvedilol in diabetic rats, prevention of defective peripheral nerve perfusion and conduction velocity. *Nauyn Schmiedbergs Arch. Pharmacol.* (1995), 351, 630 – 35.

5 Craven P.A., DeRubertis F.R., Kagan V.E., Melhem M., Studer R.K., Effects of supplementation with vitamin C or E on albuminuria, glomerular TGF- β 1 and glomerular size in diabetes. *Jour. Amer. Soc. Nephrol.* (1997), 8, 1405 – 11.

10 Cui, J; Holmes, EH; Greene, TG; Liu, PK.Oxidative DNA damage precedes DNA fragmentation after experimental stroke in rat brain. *Faseb Journal* (2000) May, 14(7):955-67.

Dawson G., Glaser P. Apparent cathepsin B deficiency in neuronal ceroid lipofuscinosis can be explained by peroxide inhibition. *Biochem. Biophys. Res. Commun.* (1987), 147, 267 – 74.

15 Dawson G., Glaser P. Abnormal cathepsin B activity in Batten's disease. *Amer. Jour. Med. Genet. Suppl.* (1988), 5, 209 - 20.

20 Domingo J.L., Gomez M., Sanchez D.J., Llobet JM., Keen, C.L. Toxicology of vanadium compounds in diabetic rats: the action of chelating agents on vanadium accumulation. *Molec. Cell. Biochem.* (1995), Dec 6-20, 153(1-2):233-40.

Dreyer E.B., Pan Z., TORM s., Lipton S.A. Greater sensitivity of larger retinal ganglion cells to NMDA-mediated cell death. *NeuroReport* (1994), 5, 629 - 31.

Dreyer E.B., Zurakowski D., Schumer R.A., Podos S.M., Lipton S.A. Elevated glutamate levels in the vitreous body of humans and monkeys with glaucoma. Arch. Ophthalmol. (1996), 114, 299 – 305.

5 Duara R., Lopez-Alberola R.F., Barker W.W. A comparison of familial and sporadic Alzheimer's disease. Neurology. (1993), Jul;43(7):1377-84.

Dubin D.T. Evidence for conjugation between glutathione and polyamines in E. Coli. Biochem. Biophys. Res. Commun. (1959), 1 (5), 262 - 65.

10 Dyer G.D., Dunn J.A., Thorpe S.R., Accumulation of Maillard reaction products in skin collagen I diabetics and aging. Jour. Clin. Invest. . (1993), Jun, 91(6):2463-9.

15 Earle K.E., Archer A.G., Baillie, JE. Circulating and excreted levels of chromium after an oral glucose challenge: influence of body mass index, hypoglycemic drugs, and presence and absence of diabetes mellitus. Amer. Jour. Clin. Nutr. (1989), Apr, 49(4):685-9.

20 Ebihara I., Nakamura T., Shimada N., Koide, H. Increased plasma metalloproteinase-9 concentrations precede development of microalbuminuria in non-insulin-dependent diabetes mellitus. [Comment In: Am J Kidney Dis. (1998), Oct;32(4):669-71] Amer. Jour. Kidney Dis. (1998) Oct, 32(4):544-50.

Edelstein C., Kaiser M., Piras G., Scanu A. Demonstration that the enzyme that converts precursor of apolipoprotein A-1 is secreted by the hepatocarcinoma cell line hep G2. Arch. Biochem. Biophys. (1988), 267, 23 - 30.

5 Edland S.D., Silverman J. Peskind E.R., Tsuan G.D., Wijsman E., Morris J.C. Increased risk of dementia in mothers of Alzheimer's disease cases: evidence for maternal inheritance. Neurology (1996), Jul;47(1):254-6.

10 Egawhary, DN; Swoboda, BE; Chen, J; Easton, AJ; Vince, FP. Diabetic complications and the mechanism of the hyperglycaemia-induced damage to the mt DNA of cultured vascular endothelial cells: (I) Characterization of the 4977 base pair deletion and 13 bp flanking repeats. Biochemical Soc. Trans. (1995), Nov, 23(4), 518S.

15 Eizirik D.L., Welsh N., Niemann A., Velloso L.A., Malaisse W.J. Succinic acid monomethyl ester protects rat pancreatic islet secretory potential against interleukin-1 β (IL-1 β) without affecting glutamate decarboxylase expression or nitric oxide production. FEBS Lett. (1994), 337, 298 - 302.

20 Englander, EW; Greeley, GH Jr; Wang, G; Perez-Polo, JR; Lee, HM. Hypoxia-induced mitochondrial and nuclear DNA damage in the rat brain. Jour. Neurosci. Res. (1999), Oct 15, 58(2):262-9.

Failla, M.L., Kiser R.A. Altered tissue content and cytosol distribution of trace metals in experimental diabetes. Jour. Nutr. (1981), Nov, 111(11):1900-9.

5 Fantus I.G., Tsiani E. Multifunctional actions of vanadium compounds on insulin signaling pathways: evidence for preferential enhancement of metabolic versus mitogenic effects. Molec. Cell Biochem. (1998), May 182(1-2):109-19.

Fawcett, T.G., Rudich, S.M., Toby, B.H., LaLancette, R.A., Potenza, J.A., Schugar, H.J. Studies of Chelation Therapy. Inorg. Chem. (1980), 19, 940.

10 Ferrari, R. The role of mitochondria in ischemic heart disease. Jour. Cardiovasc. Pharmacol. (1996), 28 Suppl 1, S1-10.

15 Fischer, H.R., Hodgson, D.J., Michelsen, K., Pedersen, E., Synthesis and Characterization of the Dimeric Cr(III) Complex Di--hydroxobis[{N,N'-bis(2-pyridylmethyl)-1,3-propanediamine}chromium (III)] Perchlorate, Inorg. Chim. Acta, (1984), 88, 143-150.

Freiberg J.M., Schneider T.R., Streeten D.H.A. Effect of Brewer's yeast on glucose tolerance. Ann. Meeting Amer. Diab. Assoc. (1975) Abstract.

20 Gai W.P., Blumbergs P.C., Blesing W.W. The ultrastructure of Lewy neurites. Mov. Disord. (1977), 12, Supl.1:5.

Gerbitz, KD. Does the mitochondrial DNA play a role in the pathogenesis of diabetes?
Diabetologia (1992), Dec, 35(12), 1181-6.

Gilad G., Dornay M., Gilad V. Polyamines induce precocious development in rats. Possible
interaction with growth factors. Int. Jour. Devl. Neurosci. (1989), 7(6), 641 - 53.

Goering P.L., Tandon S.K., Klaassen C.D. Induction of hepatic metallothionein in mouse liver
following administration of chelating agents. Toxicol. Appl. Pharmacol. (1985), 80, 467 - 72.

Goldfine A.B., Simonson D.C., Folli F., Patti, ME., Kahn C.R. In vivo and in vitro
studies of vanadate in human and rodent diabetes mellitus. Molec. Cell. Biochem.
(1995) Dec 6-20, 153(1-2):217-31.

Goldfine A.B., Patti M.E., Zuberi L., Goldstein B.J., LeBlanc R., Landaker E.J., Jiang Z.Y.,
Willsky G.R., Kahn C.R.. Metabolic effects of vanadyl sulfate in humans with non-insulin-
dependent diabetes mellitus: in vivo and in vitro studies. Metabolism: Clin. Experim. (2000)
Mar, 49(3):400-10.

Golub, G., Cohen, H., Meyerstein, D., The Stabilization of Monovalent Copper Ions by
Complexation with Saturated Tertiary Amine Ligands in Aqueous Solutions. Jour. Chem.
Soc., Chem. Commun., (1992), 397-398.

Goodwin, H.A., Lions, F., Quadridentate Chelate Compounds, Jour. Amer. Chem. Soc.,
(1960), 82, 5013-5023.

Goodman Y., Bruce A.J., Cheng B., Mattson M.P., Estrogens attenuate and corticosterone exacerbates excitotoxicity, oxidative injury and amyloid β -peptide toxicity in hippocampal neurons. Jour. Neurochem. (1996), 66, 1836 – 44.

5 Grankvist, K; Marklund, SL. Opposite effects of two metal-chelators on alloxan-induced diabetes in mice. Life Sci. (1983), Dec 19, 33(25):2535-40.

Hagglof B., Hallmans G., Holmgren G., Ludvigsson J., Falkmer S. Prospective and retrospective studies of zinc concentration in serum, blood clots, hair and urine in young patients with insulin-dependent diabetes mellitus. Acta Endocrin. (Copenh) (1983), 102, 88 – 95.

Haglund, B; Ryckenberg, K; Selinus, O; Dahlquist, G. Evidence of a relationship between childhood-onset type I diabetes and low groundwater concentration of zinc. Diabetes Care 1996 Aug, 19(8):873-5.

15 Hamakubo T., Kannagi R., Murachi T., Matus A. Distribution of calpain I and II in rat brain. Jour. Neurosci. (1986), 6, 3103 - 11.

20 Hambidge, KM; Rodgerson, DO; O'Brien, D. Concentration of chromium in the hair of normal children and children with juvenile diabetes mellitus. Diabetes 1968 Aug, 17(8):517-9.

24 Hay, R.W., Gidney, P.M., Lawrance, G.A., Cobalt Complexes of 3,7-Dithianonane-1,9-diamine. Jour. Chem. Soc., Dalton, (1975), 779-784.

Heyliger C.E., Tahiliani A.G., McNeill J.H. Effect of vanadate on elevated blood glucose and depressed cardiac performance of diabetic rats. *Science* (1985), Mar 22, 227(4693):1474-7.

Husain S., Hadi S.M. Strand scission in DNA induced by L-DOPA in the presence of Cu(II). *FEBS Lett.* (1995), 364, 75 – 78.

Husain S., Hadi S.M. DNA breakage by L-DOPA and Cu(II): breakage by melanin and bacteriophage inactivation. *Mutat. Res.* (1998), 397, 161 – 68.

Isobe, K; Ito, S; Hosaka, H; Iwamura, Y; Kondo, H; Kagawa, Y; Hayashi, JI. Nuclear-recessive mutations of factors involved in mitochondrial translation are responsible for age-related respiration deficiency of human skin fibroblasts. *Jour. Biol. Chem.* (1998), Feb 20, 273(8):4601-6.

Jiang Z.Y., Zhou Q.L., Eaton J.W., Koppenol W.H., Hunt J.V., Wolff S.P. Spirohydantoin inhibitors of aldose reductase inhibit iron- and copper-catalysed ascorbate oxidation in vitro. *Biochem. Pharmacol.* (1991), Aug 22, 42(6):1273-8.

Johns, DR, Sadun, AA, Cuban epidemic optic neuropathy. Mitochondrial DNA analysis. *Jour. Neuro-Ophthalmol.*, (1994), Sep, 14(3):130-4.

Karasu C., Dewhurst M., Stevens E.J., Tomlinson D.R., Effects of anti-oxidant treatment on sciatic nerve dysfunction in streptozotocin-diabetic rats; comparison with essential fatty acids. *Diabetologia* (1995), 38, 129 – 34.

Kelner M.J., Bagnell R., Hale B., Alexander N.M. Inactivation of intracellular copper-zinc superoxide dismutase by chelating agents without glutathione depletion and methemoglobin formation. *Free Radical Biol. Med.* (1989), 6 (4), 355 - 60.

5 Khan A.U. , DiMascio P., Medeiros M. H., Wilson T. Spermine and spermidine protect ion of plasmid DNA against single-strand breaks induced by singlet oxygen. *Proc. Natl. Acad. Sci.* (1992), 89, 11428 - 30.

Kinlaw W.B., Levine A.S., Morley J.E., Silvs S.E., McClain C.J. Abnormal zinc metabolism in type II diabetes mellitus. *Amer. Jour. Med.* (1983), 75, 273 - 7.

Kodama, H; Murata, Y; Iitsuka, T; Abe, T. Metabolism of administered triethylene tetramine dihydrochloride in humans. *Life Sciences*, (1997), 61(9):899-907.

15 Kooistra T., Van Hinsbergh V., Havekes L., Jan Kempen H. In vitro studies on origin and site of action of enzyme activity responsible for conversion of human proapoprotein A-1 into apoprotein A-1. *FEBS Lett.* (1984), 170, 109 - 13.

Kristal B.S., Koopmans S.J., Jackson Y., Ikeno B.J., Park B.J., Yu B.P. Oxidant-mediated repression of mitochondrial transcription in diabetic rats. *Free Radical Biol. Med.* (1997), 813 - 22.

Kruman, II; Culmsee, C; Chan, SL; Kruman, Y; Guo, Z; Penix, L; Mattson, M.P. Homocysteine elicits a DNA damage response in neurons that promotes apoptosis and hypersensitivity to excitotoxicity. *Journal of Neuroscience* (2000), Sep 15, 20(18):6920-26.

5 Krumkalns, E.V., Pfeifer, W., Adamantylamines by Direct Amination of 1-Bromoadamantane, *Jour. Med. Chem.*, (1968), 11, 1103.

Langsjoen, PH; Folkers, K; Lyson, K; Muratsu, K; Lyson, T; Langsjoen, P. Effective and safe therapy with coenzyme Q10 for cardiomyopathy. *Klinische Wochenschrift* (1988), Jul 1, 66(13):583-90.

Leclercq-Meyer V., Malaisse W.J., Enhancement by succinic acid dimethylester of insulin release evoked by D-glucose and glimepiride in the perfused pancreas of normoglycemic and hyperglycemic rats. *Biochem. Pharmacol.* (1993), 47, 1519 – 24.

15 Lee T.S., Saltsman K.A., Ohashi H. Activation of protein kinase C by elevation of glucose concentration: proposal for a mechanism in the development of diabetic vascular complications. *Proc. Natl. Acad. Sci.* (1989), Jul; 86(13):5141-5.

20 Lee, HK; Song, JH; Shin, CS; Park, DJ; Park, KS; Lee, KU; Koh, CS. Decreased mitochondrial DNA content in peripheral blood precedes the development of non-insulin-dependent diabetes mellitus. *Diabetes Res.Clin. Prac.* (1998) Dec, 42(3):161-7.

Levay G., Ye Q., Bodell W.J. Formation of DNA adducts and oxidative base damage by copper mediated oxidation of dopamine and 6-hydroxydopamine. *Exper. Neurol.* (1997), 146, 570 – 74.

Lovell M.A., Robertson J.D., Teesdale W.J., Campbell J.L., Markesbery W.R. Copper, iron, and zinc in Alzheimer's disease senile plaques. *Jour. Neur. Sci.* (1998), 158, 47 – 52.

Lynch J.J. Ferro T.J., Blumenstock F.A. Increased endothelial albumin permeability mediated by protein kinase C activation. *Jour. Clin. Invest.* (1990), Jun;85(6):1991-98.

Low, PA; Nickander, KK; Tritschler, HJ. The roles of oxidative stress and antioxidant treatment in experimental diabetic neuropathy. *Diabetes* (1997), Sep, 46 Suppl 2:S38-42.

MacDonald M.J., Fahien L.A. Glyceraldehyde phosphate and methyl esters of succinic acid. Two "new" potent insulin secretagogues. *Diabetes.* (1988), Jul;37(7):997-9.

Malaisse W.J, Sener A. Metabolic effects and fate of succinate esters in pancreatic islets. *Amer. Jour. Physiol.*(1993), Mar;264(3 Pt 1):E434-40.

Malaisse W.J., Rassachaert J., Villaneuva-Penacarrillo M.I., Valverde I. Respiratory, ionic, and functional effects of succinate esters in pancreatic islets. *Amer. Jour. Physiol.* (1993), Mar;264(3 Pt 1):E428-33.

Malaisse, WJ. The β cell in NIDDM: giving light to the blind. *Diabetologia* (1994), Sep, 37 Suppl 2:S36-42.

Matus A., Green G. Age-related increase in a cathepsin D like protease that degrades brain microtubule-associated proteins. *Biochemistry* (1987), 26, 8083 - 86.

Maechler, P; Wollheim, CB. Mitochondrial signals in glucose-stimulated insulin secretion in the β cell. *Jour. Physiol.* (2000) Nov 15, 529 Pt 1:49-56.

Maedler, K; Spinas, GA; Dyntar, D; Moritz, W; Kaiser, N; Donath, MY. Distinct effects of saturated and monounsaturated fatty acids on β -cell turnover and function. *Diabetes* (2001), Jan, 50(1):69-76.

Marin-Garcia, J; Goldenthal, MJ; Ananthakrishnan, R; Pierpont, ME. The complete sequence of mtDNA genes in idiopathic dilated cardiomyopathy shows novel missense and tRNA mutations. *Jour. Card. Fail.* (2000), Dec, 6(4), 321-9.

Mateo M.C.M., Bustamante J.B., Cantalapiedra M.A.G. Serum zinc, copper and insulin in diabetes mellitus. *Biomed.* (1978), 29, 56 - 58.

Mattson, MP; Culmsee, C; Yu, ZF. Apoptotic and antiapoptotic mechanisms in stroke. *Cell Tissue Res.* (2000), Jul, 301(1):173-87.

McCance D.R., Dyer D.G., Dunn J.A., Bailie K.E., Thorpe S.R., Baynes J.W., Lyons T.J.
.Maillard reaction products and their relation to complications in insulin-dependent diabetes
mellitus. Jour. Clin. Invest. (1993), Jun 91(6):2470-8.

5 McCully K.S. Homocysteine metabolism in scurvy, growth and arteriosclerosis. Nature
(1971), 23, 391 – 92.

McCully K.S., Vezeridis M.P., Histopathological effects of homocysteine thiolactone on
epithelial and stromal tissues. Exp. Molec. Pathol. (1989), 51, 159 – 70.

10 McCully K.S., Olszewski A.J., Vezeridis M.P. Homocysteine and lipid metabolism in
atherogenesis: effect of the homocysteine thiolactonyl derivatives, thioretinaco and
thioretinamide. Atherosclerosis (1990), Aug, 83(2-3):197-206.

15 McCully, K.S. Tzanakakis G.N., Vezeridis M.P. Effect of the synthetic N-homocysteine
thiolactonyl derivatives, thioretinaco, thioretinamide, and thioco on growth and lactate
production by malignant cells. Res. Commun. Chem. Pathol. Pharmacol. (1992), Jul,
77(1):125-8.

20 McCully K.S. Chemical pathology of homocysteine. III. Cellular function and aging. Ann.
Clin. Lab. Sci. (1994a), Mar-Apr, 24(2):134-52.

24 McCully, KS. Chemical pathology of homocysteine. II. Carcinogenesis and homocysteine
thiolactone metabolism. Ann. Clin. Lab.Sci. (1994b), Jan-Feb, 24(1):27-59.

McGurk J.F., Bennett M.V., Zukin R.S. Polyamines potentiate responses of N-methyl-D-aspartate receptors expressed in *Xenopus* oocytes. *Proc. Natl. Acad. Sci.* (1990), 87, 9971 - 74.

McLaren G.D. Muir WA. Kellermeyer R.W. Iron overload disorders: natural history, pathogenesis, diagnosis and therapy. *Crit. Rev. Clin. Lab. Sci.* (1983), 19, 205 - 266.

Mecocci, P; Polidori, MC; Ingegneri, T; Cherubini, A; Chionne, F; Cecchetti, R; Senin, U. Oxidative damage to DNA in lymphocytes from AD patients. *Neurology* (1998), Oct, 51(4):1014-7.

Mecocci P., MacGarvey U., Kaufman A.E. Oxidative damage to mitochondrial DNA shows marked age dependent changes increases in human brain. *Ann. Neurol* (1993), 34, 609 - 16.

Mecocci, P; MacGarvey, U; Beal, MF. Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. *Annals Neurol.* (1994), Nov, 36(5):747-51.

Mezzetti G., Monti M.G., Moruzzi M.S. Polyamines and the catalytic domain of protein kinase C. *Life Sci.* (1988), 42, 2293 - 98.

Mizukami, F., Metal Complexes Containing Six-Membered Chelate Rings. The Preparation and Structure of Dichlorocobalt(III) Complexes with Tetramines Derived from 2,4-Pentanedimine, *Bull. Chem. Soc. Jpn.*, (1975), 48, 1205-1212.

Mikukami, F., Metal Complexes Containing Six-Membered Chelate Rings. IV. The Preparation and Structure of Dichlorocobalt(III) Complexes with Tetramines Derived from 2,4-Pentanediamine. Bull. Chem. Soc, Japn., (1975), 48, 1205-1212.

5 Miyako, K; Kai, Y; Irie, T; Takeshige, K; Kang, D. The content of intracellular mitochondrial DNA is decreased by 1-methyl-4-phenylpyridinium ion (MPP+). Jour. Biol. Chem. (1997), Apr 11, 272(15):9605-8.

10 Miyako, K; Irie, T; Muta, T; Umeda, S; Kai, Y; Fujiwara, T; Takeshige, K; Kang, D. 1-Methyl-4-phenylpyridinium ion (MPP+) selectively inhibits the replication of mitochondrial DNA. Eur. Jour. Biochem. (1999), Jan, 259(1-2):412-8.

15 de la Monte, SM; Luong, T; Neely, TR; Robinson, D; Wands, JR. Mitochondrial DNA damage as a mechanism of cell loss in Alzheimer's disease. Lab. Invest. (2000), Aug, 80(8):1323-35.

Morgan J.M. Hepatic chromium content in diabetic subjects. Metabolism: Clin. Exper. (1972), Apr, 21(4):313-6.

20 Moruzzi M.S, Monti M.G., Piccinini G., Marverti G., Tadolini B. Effect of spermine on association of protein kinase C with phospholipid vesicles. Life Sci. (1990), 47(16), 1475 - 82.

Moruzzi M.S., Marverti G., Piccinini G., Frassinetti C., Monti M.G. The effect of spermine on calcium requirement for protein kinase C association with phospholipid vesicles. *International Jour. Biochem. Cell Biol.* (1995), 27(8), 783 - 8.

Moruzzi M.S., Piccinini G., Tadolini B., Monti M.G., Barbiroli B., Mezzetti G. In: Progress in polyamine research. Effect of polyamines on protein kinase C activation process. *Adv. Exp. Med. Biol.* (1988), 250:469-80.

Moustaid N., Sul H.S., Regulation of expression of the fatty acid synthase gene in 3T3-L1 cells by differentiation and triiodothyronine. *Jour. Biol. Chem.* (1991), 18550 – 554.

Naviaux, RK. Mitochondrial DNA disorders. *Eur. Jour. Ped.* (2000), Dec, 159 Suppl 3:S219-26.

Noto R., Alicata R., Sfogliano L. A study of cupremia in a group of elderly diabetics. *Acta Diabetol. Latina* (1983), 20, 81- 85.

Oberholzer, M.R., Neuburger, M., Zehnder, M., Kaden, T.A., Steric Effects in the Cu(II) and Ni(II) Complexes with Tetra-N-alkylated 1,4,8,11-Tetraazacyclotetradecanes. *Helv. Chim Acta*, (1995), 78, 505.

Odawara, M; Yamashita, K. Mitochondrial gene abnormalities and α - and β -cell dysfunction. *Diabetes Care* (1996), Oct, 19(10):1166-7.

10017235-121801

Oka Y., Katagiri H., Yazaki Y., Murase T., Kobayashi T. Mitochondrial gene mutation in islet-autoantibody-positive patients who were initially non-insulin-dependent diabetics. *Lancet* (1993), 342, 527 – 28.

Oka, Y. NIDDM--genetic marker; glucose transporter, glucokinase, and mitochondria gene. *Diabetes Res. Clin. Prac.* (1994), Oct, 24 Suppl., S117-21.

Ozawa, T. Mitochondrial DNA mutations in myocardial diseases. *European Heart Journal* (1995), Dec, 16 Suppl O, 10-14.

Ozawa, T; Hayakawa, M; Katsumata, K; Yoneda, M; Ikebe, S; Mizuno, Y. Fragile mitochondrial DNA: the missing link in the apoptotic neuronal cell death in Parkinson's disease. *Biochem. Biophys. Res. Commun.* (1997), Jun 9, 235(1):158-61.

Olszewski A.J., McCully K.S. Homocysteine metabolism and the oxidative modification of proteins and lipids. *Free Rad. Biol. Med.* (1993), Jun, 14(6):683-93.

Papadopoulou, LC; Theophilidis, G; Thomopoulos, GN; Tsiftoglou, AS. Structural and functional impairment of mitochondria in adriamycin-induced cardiomyopathy in mice: suppression of cytochrome c oxidase II gene expression. *Biochem. Pharmacol.* (1999), Mar 1, 57(5):481-9.

Park, KS; Lee, KU; Song, JH; Choi, CS; Shin, CS; Park, DJ; Kim, SK; Koh, JJ; Lee, HK. Peripheral blood mitochondrial DNA content is inversely correlated with insulin secretion

during hyperglycemic clamp studies in healthy young men. *Diabetes Res. Clin. Prac.* (2001), May, 52(2):97-102.

Perlmutter L.S., Siman R., Gall C., Seubert P., Lynch G. The ultrastructural localization of calcium- activated protease "calpain" in rat brain. *Synapse* (1988), 2, 78 - 88.

5

Press E.M., Porter R., Cebra J. The isolation and properties of a proteolytic enzyme, cathepsin D, from bovine spleen. *Biochem. Jour.* (1960), 74, 501 - 14.

Quigley H.A. Ganglion cell death in glaucoma: Pathology recapitulates ontogeny. *Aust. NZ J. Ophthalmol.* (1995), 23, 85 - 91.

Razzaboni B.L., Papastoitsis G., Koo E.H., Abraham C.R., A calcium-stimulated serine protease from monkey brain degrades the B-amyloid precursor protein. *Brain Res.* (1992), 589, 207 - 16.

15

Reul, B.A., Amin S.S., Buchet, J.P., Ongemba L.N., Crans D.C., Brichard S.M. Effects of vanadium complexes with organic ligands on glucose metabolism: a comparison study in diabetic rats. *Brit. Jour. Pharmacol.* (1999) Jan, 126(2):467-77.

20

Rose C., Camus A., Schwartz J.C. A serine peptidase responsible for the inactivation of endogenous cholecystokinin in brain. *Proc. Natl. Acad. Sci.* (1988), 85(21), 8326 - 30.

Rose C., Camus A., Schwartz J. Protection by serine peptidase inhibitors of endogenous cholecystokinin released from brain slices. *Neuroscience* (1989), 29 (3), 583 - 94.

24

Sadun, A. Acquired mitochondrial impairment as a cause of optic nerve disease. Trans. Amer. Ophthalmol. Soc. (1998), 96:881-923.

Scanu A.M. Proapolipoprotein-converting enzymes and high density lipoprotein early events in biogenesis. Amer. Heart Jour. (1987), 113, 527 - 33.

Schapira, AH. Evidence for mitochondrial dysfunction in Parkinson's disease--a critical appraisal. Movement Disorders (1994), Mar., 9(2):125-38.

Schapira, AH. Mitochondrial dysfunction in neurodegenerative disorders. Biochim. Biophys. Acta (1998), Aug 10, 1366(1-2):225-33.

Schmitz C; Axmacher B; Zunker U; Korr H. Age-related changes of DNA repair and mitochondrial DNA synthesis in the mouse brain. Acta Neuropath. (1999), Jan, 97(1):71-81.

Schroeder H.A. Cadmium, chromium, and cardiovascular disease. Circulation 1967 Mar, 35(3):570-82.

Schroeder H.A. Chromium deficiency as a factor in atherosclerosis. Jour. Chronic Dis. (1970), 23, 123 - 42.

Seidman MD; Khan MJ; Bai U; Shirwany N; Quirk WS. Biologic activity of mitochondrial metabolites on aging and age-related hearing loss. Amer. Jour. Otol. (2000), Mar, 21(2):161-7.

Serradas P., Girox M.H., Saulnier C., Mitochondrial deoxyribonucleic acid content is specifically decreased in adult but not fetal pancreatic islets of the Goto-Kakizaki rat, a genetic model of insulin-dependent diabetes. *Endocrinology* (1995), 136, 5623 – 31.

Shoffner, JM; Brown, MD; Torroni, A; Lott, MT; Cabell, MF; Mirra, SS; Beal, MF; Yang, CC; Gearing, M; Salvo, R; et al. Mitochondrial DNA variants observed in Alzheimer disease and Parkinson disease patients. *Genomics* (1993), Jul, 17(1):171-84.

Smith M.A., Harris P.L., Sayre L.M., Perry G. Iron Accumulation in Alzheimer's disease is a source of redox-generated free radicals. *Proc. Natl. Acad. Sci.* (1997), 94, 9866 – 9868.

Snyder R.D. Inhibition of X-ray-induced DNA strand break repair in polyamine depleted HeLa cells. *Int. Jour. Radiat. Biol.* (1989), 15(5), 773 – 79.

Soong N.W., Hinton D.R., Cortopassi G., and Arnheim N. Mosaicism for a specific somatic mitochondrial DNA mutation in adult human brain. *Nat. Genet.* (1992), Dec;2(4):318-23.

Strout H.V., Vicario P.P., Biswas C., Saperstein R., Brady E.J., Pilch P.F., Berger J. Vanadate treatment of streptozotocin diabetic rats restores expression of the insulin responsive glucose transporter in skeletal muscle. *Endocrin.* (1990), 126, 2728 – 32.

Sucher N.J., Lipton S.A., Dreyer E.B., (1997), Molecular pathology of glutamate toxicity in retinal ganglion cells. *Vision Res.* (1997), 37, 3483 – 93.

Suzuki Y., Kadowaki H., Atsumi Y., A case of diabetic amyotrophy associated with 3243 mitochondrial tRNA (Leu UUR) mutation and successful therapy with coenzyme Q10. Endoc. Jour. (1995), 42, 141 – 45.

5 Suzuki S; Hinokio Y; Komatu K; Ohtomo M; Onoda M; Hirai S; Hirai M; Hirai A; Chiba M; Kasuga S; Akai H; Takahashi T., Hiratani K, Kimura E., N₂-Binding Mononuclear Ru(II) Tertiary Polyamine Complex, Chemistry Letters, (1993), 1329-1332.

10 Swoboda BE; Egawhary DN; Chen J; Vince FP. Diabetic complications and the mechanism of the hyperglycaemia-induced damage to the mt DNA of cultured vascular endothelial cells: (II) The involvement of protein kinase C. Biochem. Soc. Trans. (1995), Nov, 23(4):519S.

15 Tagami M., Ikeda K., Yamagata K., Nara Y., Fujino H., Kubota A., Numano F., Yamori Y. Vitamin E prevents apoptosis in hippocampal neurons caused by cerebral ischemia and reperfusion in stroke-prone spontaneously hypertensive rats. Lab. Invest. (1999), 79, 609 – 15.

20 Tasker R.C., Sahota S.K., Cotter F.E., Williams S.R. Early post-ischemic dantrolene induced amelioration of poly(ADP-ribose) polymerase-related bioenergetic failure in neonatal rat brain slices. Jour. Cereb. Blood Flow Metab. (1998), 18, 1346 – 56.

24 Toyota, T. Oxidative damage to mitochondrial DNA and its relationship to diabetic complications. Diabetes Res. Clin. Prac. (1999), Sep, 45(2-3):161-8.

Trachtman H., Futterweit S., Maesake J. Taurine ameliorates chronic streptozotocin-induced diabetic nephropathy in rats. *Amer. Jour. Physiol.* (1995), 269, F429 – F438.

5 Tsuji A., Sakurai H. Vanadyl ion suppresses nitric oxide production from peritoneal macrophages of streptozotocin-induced diabetic mice. *Biochem. Biophys. Res. Commun.* (1996), Sep 13, 226(2):506-11.

10 Uemura, S; Matsushita, H; Li, W; Glassford, AJ; Asagami, T; Lee, KH; Harrison, DG; Tsao, PS. Diabetes mellitus enhances vascular matrix metalloproteinase activity: role of oxidative stress. *Circul. Res.* (2001), Jun 22, 88(12):1291-8.

15 Umeda S; Muta T; Ohsato T; Takamatsu C; Hamasaki N; Kang D. The D-loop structure of human mtDNA is destabilized directly by 1-methyl-4-phenylpyridinium ion (MPP+), a Parkinsonism-causing toxin. *Eur. Jour. Biochem.* (2000), Jan, 267(1):200-6.

Uriu-Hare J.Y., Stern, J.S., Keen C.L. The effect of diabetes on the molecular localization of maternal and fetal zinc and copper metalloprotein in the rat. *Biol. Trace Element Res.* (1988), Dec, 18:71-9.

20 Van Alphen J. On aliphatic polyamines III. *Jour. Rec. Trav. Chim.* (1936), 55, 835 - 40.

24 Valera A., Rodriguez-Gil J.E., Bosch F. Vanadate treatment restores the expression of genes for key enzymes in the glucose and ketone bodies metabolism in the liver of diabetic rats. *Jour. Clin. Invest.* (1992), 92, 4 –11.

Vicent D., Villaneuva-Penacarillo M.L., Valverde I., Malaisse W.J. enhancement of the insulinotropic action of glibenclamide by succinnic acid methyl esters in anaesthetized rats. Med. Sci. Res. (1993), 21, 517 – 18.

Wallace, D.C. Mitochondrial genetics: a paradigm for aging and degenerative diseases? Science (1992), May 1, 256(5057):628-32.

Wei Y.H., Kao S.H. Mitochondrial DNA mutations and lipid peroxidation in human aging. In: Nutrients and Gene Expression. Berdanier C.D. ed. Boca Raton FL: CRC Press, (1996); pp 165 – 88.

Whitaker J.N., Seyer J. Isolation and characterization of bovine brain cathepsin D. Jour. Neurochem. (1979), 32, 325 - 33.

Yang, J; Cherian, MG. Protective effects of metallothionein on streptozotocin-induced diabetes in rats. Life Sci. (1994), 55(1):43-51.

Yoshimoto, S; Sakamoto, K; Wakabayashi, I; Masui, H. Effect of chromium administration on glucose tolerance in stroke-prone spontaneously hypertensive rats with streptozotocin-induced diabetes. Metabolism: Clin. Exper.(1992), Jun, 41(6):636-42

Yu Z.F., Bruce-Keller A.J., Goodman Y., Mattson M.P. Uric acid protects neurons against excitotoxic and metabolic insults in cell culture, and against focal ischemic brain injury in vivo. Jour. Neurosci. Res. (1998), 53, 613 – 25.

Zeviani, M; Mariotti, C; Antozzi, C; Fratta, GM; Rustin, P; Prella, A. OXPHOS defects and mitochondrial DNA mutations in cardiomyopathy. Muscle and Nerve (1995), 3, S170-4.

Zeviani M., Amati P., Comi G., Fratta G., Mariotti C., Tiranti V. Searching for genes affecting the structural integrity of the mitochondrial genome. Biochim. Biophys. Acta (1995), 1271, 153 – 58.

10017233-121801